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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).



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| INVENTOR(S) | | |
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*Additional inventors are being named on the _____ separately numbered sheets attached hereto*

**TITLE OF THE INVENTION (500 characters max)**

TRANSCRIPTIONAL REGULATORY CODES OF EUKARYOTIC GENOMES AND METHODS THEREOF

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

[X] Customer Number: 28120

***OR***

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**ENCLOSED APPLICATION PARTS (*check all that apply*)**

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|---|---|---|---|
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| [X] Application Data Sheet. See 37 CFR 1.76 | | | |

**METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT**

[ ] Applicant claims small entity status. See 37 CFR 1.27.

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No [ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____



*Respectfully submitted,*

Date March 4, 2004

SIGNATURE Ignacio Perez de la Cruz

TYPED OR PRINTED NAME Ignacio Perez de la Cruz

REGISTRATION NO. *(if appropriate)* 55,535

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Docket Number: WIBL-P60-035

***USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT***

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Dated: March 4, 2004 Signature: Ignacio Perez de la Cruz (Ignacio Perez de la Cruz)

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# FEE TRANSMITTAL for FY 2004

*Effective 10/01/2003, Patent fees are subject to annual revision.*

☐ Applicant claims small entity status. See 37 CFR 1.27

**TOTAL AMOUNT OF PAYMENT** ($) 160.00

| Complete if Known | |
|---|---|
| Application Number | Not Yet Assigned |
| Filing Date | March 4, 2004 |
| First Named Inventor | Christopher T. Harbison |
| Examiner Name | Not Yet Assigned |
| Art Unit | N/A |
| Attorney Docket No. | WIBL-P60-035 |

## METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit Card ☐ Money Order ☐ Other ☐ None

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**The Director is authorized to:** *(check all that apply)*

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☒ Charge any additional fee(s) or any underpayment of fee(s)

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## FEE CALCULATION

### 1. BASIC FILING FEE

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description | Fee Paid |
|---|---|---|---|---|---|
| 1001 | 770 | 2001 | 385 | Utility filing fee | |
| 1002 | 340 | 2002 | 170 | Design filing fee | |
| 1003 | 530 | 2003 | 265 | Plant filing fee | |
| 1004 | 770 | 2004 | 385 | Reissue filing fee | |
| 1005 | 160 | 2005 | 80 | Provisional filing fee | 160.00 |

**SUBTOTAL (1)** ($) 160.00

### 2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

| | | | Extra Claims | | Fee from below | = | Fee Paid |
|---|---|---|---|---|---|---|---|
| Total Claims | | -** = | | x | | = | |
| Independent Claims | | -** = | | x | | = | |
| Multiple Dependent | | | | | | = | |

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description |
|---|---|---|---|---|
| 1202 | 18 | 2202 | 9 | Claims in excess of 20 |
| 1201 | 86 | 2201 | 43 | Independent claims in excess of 3 |
| 1203 | 290 | 2203 | 145 | Multiple dependent claim, if not paid |
| 1204 | 86 | 2204 | 43 | ** Reissue independent cleims over original patent |
| 1205 | 18 | 2205 | 9 | ** Reissue claims in excess of 20 and over original patent |

**SUBTOTAL (2)** ($) 0.00

***or number previously paid, if greater; For Reissues, see above*

## FEE CALCULATION (continued)

### 3. ADDITIONAL FEES

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description | Fee Paid |
|---|---|---|---|---|---|
| 1051 | 130 | 2051 | 65 | Surcharge – late filing fee or oath | |
| 1052 | 50 | 2052 | 25 | Surcharge – late provisional filing fee or cover sheet. | |
| 1053 | 130 | 1053 | 130 | Non-English specification | |
| 1812 | 2,520 | 1812 | 2,520 | For filing a request for *ex parte* reexamination | |
| 1804 | 920* | 1804 | 920* | Requesting publication of SIR prior to Examiner action | |
| 1805 | 1,840* | 1805 | 1,840* | Requesting publication of SIR after Examiner action | |
| 1251 | 110 | 2251 | 55 | Extension for reply within first month | |
| 1252 | 420 | 2252 | 210 | Extension for reply within second month | |
| 1253 | 950 | 2253 | 475 | Extension for reply within third month | |
| 1254 | 1,480 | 2254 | 740 | Extension for reply within fourth month | |
| 1255 | 2,010 | 2255 | 1,005 | Extension for reply within fifth month | |
| 1401 | 330 | 2401 | 165 | Notice of Appeal | |
| 1402 | 330 | 2402 | 165 | Filing a brief in support of an appeal | |
| 1403 | 290 | 2403 | 145 | Request for oral hearing | |
| 1451 | 1,510 | 1451 | 1,510 | Petition to institute a public use proceeding | |
| 1452 | 110 | 2452 | 55 | Petition to revive – unavoidable | |
| 1453 | 1,330 | 2453 | 665 | Petition to revive - unintentionel | |
| 1501 | 1,330 | 2501 | 665 | Utility issue fee (or reissue) | |
| 1502 | 480 | 2502 | 240 | Design issue fee | |
| 1503 | 640 | 2503 | 320 | Plant issue fee | |
| 1460 | 130 | 1460 | 130 | Petitions to the Commissioner | |
| 1807 | 50 | 1807 | 50 | Processing fee under 37 CFR 1.17(q) | |
| 1806 | 180 | 1806 | 180 | Submission of Information Disclosure Stmt | |
| 8021 | 40 | 8021 | 40 | Recording each patent assignment per property (times number of properties) | |
| 1809 | 770 | 2809 | 385 | Filing a submission after final rejection (37 CFR 1.129(a)) | |
| 1810 | 770 | 2810 | 385 | For each edditionel invention to be examined (37CFR 1.129(b)) | |
| 1801 | 770 | 2801 | 385 | Request for Continued Examination (RCE) | |
| 1802 | 900 | 1802 | 900 | Request for expedited examination of a design application | |
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*Reduced by Basic Filing Fee Paid

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| SUBMITTED BY | | | | (Complete *(if applicable)*) | |
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| *Signature* | Ignacio Perez de la Cruz | | | *Date* | March 4, 2004 |

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Dated: March 4, 2004 Signature: Ignacio Perez de la Cruz (Ignacio Perez de la Cruz)

Application No. (if known): Not Yet Assigned | Attorney Docket No.: WIBL-P60-035

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Ignacio Perez de la Cruz
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Application Data Sheet (3 pages)
Application (37 pages)
Drawings (38 sheets)

# Transcriptional Regulatory Codes of Eukaryotic Genomes and Methods Thereof

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Supporting Online Material

Address: http://web.wi.mit.edu/young/regulatory_code

Username: reviewer

Password: code

**We report here a first draft of a genome's transcriptional regulatory code, the set of sequences utilized by DNA-binding regulators to control genome expression programs. The code was derived by combining data on genomic binding locations for transcriptional regulators in yeast cells grown in multiple environmental conditions, knowledge of genome sequence conservation and prior evidence for regulator-DNA interactions. We discuss new insights into global transcriptional regulation that are revealed by the code, including the organization of regulatory elements in promoters and the environment-dependent use of these elements by regulators. We find that environment-specific use of the regulatory code predicts mechanistic models for the function of a large population of yeast's transcriptional regulators.**

Genome sequences contain information necessary to control gene expression programs and specify protein and other gene products. DNA-binding transcriptional regulators interpret the genome's regulatory code by binding to specific sequences to induce or repress gene expression [1-3]. Substantial portions of genome sequence are believed to be regulatory [4-8], but the DNA sequences that actually contribute to the regulatory code are ill-defined. In contrast, the triplet code used to translate nucleotide sequences into protein molecules is well known [9-11]. Knowledge of the genome's transcriptional regulatory code could provide new insights into the principles that govern global gene regulation.

Comparative genomics has recently been used to identify functional sequence elements in the yeast genome [4,5,12-14]. Comparative analysis of the genome sequences of multiple yeast species revealed phylogenetically conserved sequences, and these sequences were used to facilitate identification of genes and putative regulatory elements. Conserved sequence information alone does not reveal, however, the subset of sequences that are bound by transcriptional regulators, the identity of the binding regulators, or the conditions under which the regulators occupy their binding sites.

The set of DNA sequences that play key roles in transcriptional regulation can be deduced by identifying the genomic binding locations for individual regulatory proteins [15-20] and by searching for DNA sequences shared by the bound sites using statistical algorithms [21-23]. However, genomic binding information is available only for a subset of regulators in any organism. The genomic binding sites of 106 transcriptional regulators have been identified in yeast grown in rich medium [16], but there are approximately 200 transcriptional regulators encoded in the yeast genome, and many of these are known to function under conditions other than a rich medium environment.

**Proteome-Genome Interactions**

To elucidate a substantial portion of the yeast genome's transcriptional regulatory code, we used genome-wide location analysis [15] to determine the genomic occupancy of 203 DNA-binding transcriptional regulators in rich media conditions and, for 85 of these regulators, in at least one of twelve other environmental conditions. The 203 transcriptional regulators were identified by searching the YPD and MIPS databases [24-26] for known and predicted transcriptional regulators and nucleic acid binding proteins. These are likely to include nearly all of the DNA-binding transcriptional regulators encoded in the yeast genome. We selected regulators for profiling in a specific environment if they were essential for growth in that environment or if there was other evidence implicating them in regulation of gene expression in that environment (see online supporting data). The complete set of transcriptional regulators and the conditions under which they were profiled is listed in Supplementary Table S1, and all genome-wide location datasets are available at http://web.wi.mit.edu/young/regulatory_code.

The genome-wide location data identified 11,000 unique interactions between regulators and promoter regions. There was a broad distribution in the number of promoter regions bound by the transcriptional regulators (Figure 1a); the average regulator bound to approximately 55 promoter regions ($P < 0.001$). Among the group of regulators that bound the most promoters, a disproportionate number were nuclear regulators known to be highly abundant [27], including Abf1, Cbf1, Reb1 and Ste12. Twenty putative regulators bound no promoter regions at high confidence ($P < 0.001$), suggesting that they function under conditions that were not explored (see below) or that they are not genuine transcriptional regulators. The location data also reveal that nearly one-half of the genes that are bound by regulators were bound by two or more

regulators (Supplementary Fig. S2), and that a subset of genes are bound by a very large number of regulators, suggesting that they are regulated by multiple signals and subjected to combinatorial control.

For the 85 regulators profiled in rich media and at least one other environment, we found that a substantial number of promoter regions that were not bound in rich media were bound in the other environments. Approximately one thousand regulator-promoter region interactions identified in the other environments involved promoters that were not bound by any regulator under rich growth conditions. For example, a comparison of the promoter regions bound by 34 regulators in cells grown under rich or amino acid starved environments (Figure 1b) reveals that there are substantial differences in the number of promoters bound by many of these regulators. These results underscore the importance of the role of environmental conditions in governing DNA-regulator interactions, and we explore environment-dependent global gene regulation in more detail below.

**DNA Binding Site Sequences**

We combined genome-wide location data, phylogenetically conserved sequence information, and prior knowledge of regulator binding sequences to create a database of DNA binding sequences for transcriptional regulators (Figure 2a). To accomplish this, we first combined genome-wide location and sequence conservation data to predict binding site specificities. For regulators where we failed to predict binding site specificities at high confidence, we used evidence for binding site sequences obtained from the literature, where available.

Genome-wide location analysis identifies regions of the genome that are physically occupied by specific regulators in vivo, but does not identify the precise DNA sequences that serve as recognition sites. Numerous algorithms have been

developed to discover binding sites in a set of identified sequences. We designed a strategy to improve the yield of the discovery process by combining the results from six programs, three of which incorporate information from phylogenetic conservation [21-23,28] ; for a detailed description of the method, see supplementary data. Using this approach, we identified binding site sequence specificities for the 151 regulators that bound more than ten promoter regions. Of these, 68 met a high threshold criteria for significance (see Methods and Supplementary Table S2).

We compared the discovered binding site sequence specificities with previous knowledge by generating a list of binding sequences and sequence motifs for each regulator that was derived solely from the evidence for protein-DNA interactions as contained in the databases TRANSFAC, SCPD and YPD [24,25,29,30] (Supplementary Table S2). For 39 transcriptional regulators, we found that we identified a DNA binding site sequence that was identical to, or shared significant homology with, previously described binding sequences. For example, the DNA binding sequence motifs for Abf1, Gal4, Gcn4, Leu3 and Ste12 were rediscovered (Figure 2b). For 21 additional regulators, the DNA binding site sequence we predict was completely novel; the newly discovered sequence had not been described previously. Aft2, Rds1, Snt2, Stb4 and YDR026C were among the regulators for which novel binding sequences were discovered (Figure 2b). For 8 regulators, the DNA binding sequence we predict differs from the sequence described in the literature. This discrepancy may be due to different growth environments used in the different studies, to noise in the genome-wide location data, or to limitations in the analytical methods used here or in previous studies.

The literature suggests binding site sequences for 15 transcriptional regulators for which we did not predict a binding site sequence. We added this literature evidence to our database of DNA binding sequences for transcriptional regulators (Supplementary Table S2) and used it in subsequent analyses.

We note that the regulators bind to only a fraction of the sites in the genomic DNA that contain the predicted recognition sequence. To test whether this observation is due to the limitations of our methods or whether it reflects a fundamental aspect of genomic regulation, we investigated whether bound sites were under more selective pressure than sites that were not bound. We used DNA binding site specificities listed in the TRANSFAC database in this analysis to eliminate the sequence conservation bias inherent in the binding sites specificities determined as described above. Figure 2c shows that the genomic loci that both match specificities in the TRANSFAC database and were bound in our assay are more conserved in other *sensu stricto Saccharomyces* species than loci that match the TRANSFAC specificities but were not bound. For example, the regulator Hsf1 binds to only 23/63 genomic loci that match the TRANSFAC specificity. While 78% of the bound sequences are conserved, only 13% of the remaining sites are conserved.

There are both positive and negative regulatory mechanisms that are likely to account for the observation that DNA binding regulators bind only a subset of the sites in genomic DNA that contain that regulator's recognition sequence. Cis-acting regulatory DNA sequences frequently contain binding sites for multiple regulators that stimulate cooperative binding through protein-protein interactions or that alter local DNA structure. The presence of multiple regulators of this type might be expected to be conserved in related species, accounting for the relative selective pressure observed for the bound sequences. It is also the case that the binding of a protein to certain sites in the genome can be occluded by the presence of another protein. The proteins associated with genomic DNA include transcriptional regulators, the transcription apparatus, the DNA replication apparatus, histones and other chromatin-associated proteins, and chromatin modifying complexes. These are all likely to affect the relative occupancy of specific DNA sequences by transcriptional regulators.

We also note that some DNA binding regulators can occupy DNA sequences in the absence of a discernable binding sequence for that regulator. There are several examples of proteins that occupy specific sites in the absence of an appropriate sequence. When Tec1 is present in cells, Ste12 can be found associated with DNA with Tec1 binding sequences [31]. The Hir1/Hir2 corepressor complex, which is recruited to the promoters of histone genes, does not bind to a sequence that is conserved in each of the histone promoters [32]. The Origin of DNA Replication Complex (ORC) occupies specific genomic sites [33], but a consensus sequence that would meet the confidence criteria used here has not been found. These latter two examples demonstrate that more than one unique DNA sequence can provide a functional binding site for transcriptional and DNA synthesis regulators, and emphasize the importance of in vivo binding data in discovering functional elements in the genome.

**Transcriptional regulatory code**

Gene expression programs depend on recognition of specific DNA sequences by transcriptional regulatory proteins, so the set of DNA sequences that are bound by these proteins should reveal the genome's transcriptional regulatory code. We have constructed a draft of the yeast genome's transcriptional regulatory code by identifying the positions of the sequences that are bound by regulators in vivo in the genome sequence. To populate this draft of the regulatory code with high confidence information, we further restricted the map to include sequences that were not only bound, but were also conserved in at least two other *sensu stricto Saccharomyces* genomes [4]. Portions of the draft code is displayed in Figure 3, and the complete map can be found at the authors' website. Because the information used to construct the map includes binding data from many different growth environments, the code describes transcriptional regulatory potential within the genome.

The first level of information contained within the regulatory code is simply the association of genes with the regulators responsible for their transcriptional control. This level of information reveals insights into the control of underlying biological processes. For example, find that the promoter of *BAP2*, which encodes an extracellular amino acid transporter, is bound by the amino acid biosynthetic regulators Gcn4 and Leu3. Similarly, we identify binding of the regulator of respiration, Hap5, to a site upstream of *COX4*, a component of the respiratory electron transport chain. The ribosomal regulator Rap1 is bound to the small ribosomal subunit component gene RPS18A. Knowledge of which regulators control which genes is fundamental in understanding particular aspects of biology. However, in many cases, the identity of regulators and the arrangement of their binding sites within promoters suggests additional levels of organization (discussed below).

The distribution of binding sites for transcriptional regulators reveals there are constraints on the organization of promoters in the yeast genome (Supplemental Fig. S1). Binding sites are not uniformly distributed over the promoter regions, but rather show a sharply peaked distribution. Very few sites are located in the region 80 bp upstream of protein coding sequences. This region typically includes the transcription start site and is bound by the transcription initiation apparatus; RNA polymerase alone has been shown to occupy approximately 60 bp. The vast majority (73%) of the transcriptional regulator binding sites lie between 100 and 500 bps upstream of the protein coding sequence. It appears that yeast transcriptional regulators function better at short distances along the linear DNA, a property that reduces the potential for inappropriate activation of nearby genes and allows evolution to select for organisms that dispense with lengthy intergenic regions.

**Promoter Architectures**

We note that there are specific arrangements of DNA binding site sequences within promoters, and that these promoter architectures can provide clues to regulatory mechanisms. We identify four such distinctive arrangements (Figure 4) and discuss their biological implications below.

**Single regulator architecture.** The presence of a DNA binding site for a single regulator is the simplest promoter architecture (Figure 4). Seven hundred sixty-three promoter regions were found to have single regulator promoter architecture (see online supporting data). These promoter regions include the binding sites for 75 of the 203 regulators. We expect that sets of genes containing a binding site for a single regulator may be involved in a common function, and this is the case for genes bound by 25 regulators (Supplementary Table S3). Analysis of expression data confirmed that for 15 of these regulators, there is also strong correlation between single regulator promoters and changes in gene expression (Supplementary Table S3). We do not expect correlation in all cases since expression is known to be regulated at levels other than transcription initiation. For example, mechanisms are known that control both mRNA stability and export from the nucleus [34].

**Multiple regulator architecture.** Promoters with multiple regulator architecture contain binding sites for two or more different regulators (Figure 4). We find that 533 promoter regions contain sequences corresponding to two or more binding site motifs. These regions are bound by 99 different regulators. This promoter arrangement implies that the gene may be subject to combinatorial regulation, and we expect that in many cases the various regulators can be used to execute differential responses to different growth conditions. Indeed, we note that many of the genes in this category encode products that are required for multiple metabolic pathways and are regulated in an environment-specific fashion. For example, the promoter of *IDP1* gene, which is involved in energy production through oxidation, is bound not only by Hap4 and Rtg3,

regulators of carbon metabolism, but also by Gln3 and Gcn4. These latter proteins regulate amino acid biosynthetic pathways that require the same simple carbon compounds generated by genes under the control of Idp1.

**Repetitive motif architecture.** While some promoter regions contain only a single copy of a particular binding site sequence, others contain repeats of these sites (Figure 4). Multiple binding sites have been shown to be necessary for stable binding by the regulator Dal80 [35], and given the small size of typical binding site sequences, this requirement is likely for additional regulators. This repetitive promoter architecture can also allow for a graded transcriptional response, as has been observed for the *HIS4* gene [36,37]. The presence of repeated binding site sequences for a single regulator was observed for 71 regulators across 408 promoter regions (Figure 4). We note that a number of regulators, including Dig1, Mbp1, and Swi6 show a statistically significant preference for repetitive motifs (Supplementary Table S4).

**Co-occurring regulator architecture.** We searched the set of multiple regulator promoters to identify pairs of transcription factors whose binding sites occur more frequently within the same promoter regions than would be expected by chance (Table S5). There are ninety-three such co-occurring pairs of regulators ($P < 0.005$). We expect three types of relationships among such regulators. The first type is exemplified by the regulatory pair Gcn4 and Leu3. In this case, the pair of regulators share a functional overlap in their regulatory roles. Specifically, Gcn4 is a general regulator of amino acid biosynthetic genes whereas Leu3 regulates a smaller set of genes involved solely in the metabolism of aliphatic amino acids. This arrangement apparently allows cells the option of co-ordinately regulating either the complete set of amino acid biosynthetic genes or a specific subset. A second type of co-occurring regulator pairs consists of regulators that form physical associations. A well studied example of such a pair is Swi6 and Mbf1, which together form the MBF heterodimeric cell cycle

regulatory complex. Finally, where pairs of regulators do not interact physically, but bind to the same DNA sequence, the binding site is polysemic, conveying more than one meaning. In many cases, such regulator pairs share homologous DNA-binding domains. The regulators Fkh1 and Fkh2, for example, are known to have overlapping but distinct functions, and this is reflected in their binding profiles.

**Environment-specific Binding of Regulatory Sequences**

By conducting genome-wide binding experiments for some regulators under multiple cell growth conditions, we learned that regulator binding to a subset of the regulatory sequences is highly dependent on the environmental conditions of the cell. We observed four common patterns of regulator binding behaviour (Figure 5, Supplementary Table S6). Prior information about the regulatory mechanisms employed by well-studied regulators in each of the four groups suggests how to account for the environment-dependent binding behaviour of the other regulators.

"Condition invariant" regulators bind essentially the same set of promoters (within the limitations of noise) in two different growth environments (Figure 5). Leu3, which is known to regulate genes involved in amino acid biosynthesis, is among the best studied of the regulators in this group. Activation of Leu3-regulated genes has been shown to be independent of Leu3 binding, but requires association of a leucine metabolic precursor to convert it from negative to positive regulator [38-40]. We note that other zinc cluster type regulators that show "condition invariant" behaviour are known to be regulated in a similar manner. Thus, it is reasonable to propose that the activation or repression functions of some of the other regulators in this class will be independent of DNA binding.

"Condition enabled" regulators do not bind the genome detectably under one condition, but bind a substantial number of promoters with a change in environment.

Msn2 is among the best-studied regulators in this class, and the mechanisms involved in Msn2-dependent transcription provide clues to how the other regulators in that class may operate. Msn2 is known to be excluded from the nucleus when cells grow in the absence of stresses, but accumulates rapidly in the nucleus when cells are subjected to stress [41-43]. This condition-enabled behaviour was also observed for the thiamine biosynthetic regulator Thi2, the nitrogen regulator Gat1 and the developmental regulator Rim101. We postulate that each of these transcriptional regulators is regulated by nuclear exclusion or by another mechanism that would cause this extreme version of condition-specific binding.

"Condition expanded" regulators bind to a core set of target promoters under one condition, but bind an expanded set of promoters under another condition. Gcn4 is the best-studied of the regulators that fall into this "expanded" class. The levels of Gcn4 are reported to increase 6-fold when yeast are introduced into media with limiting nutrients [44], due largely to increased nuclear protein stability [42,45,46], and under this condition we find Gcn4 binds to an expanded set of genes. Interestingly, the probes bound when Gcn4 levels are low contain better matches to the known Gcn4 binding site than probes that are bound exclusively at higher protein concentrations, consistent with a simple model for specificity based on intrinsic protein affinity and protein concentration (Supplementary Figure S1). The expansion of binding sites by many of the regulators in this class likely reflects increased levels of the regulator available for DNA binding.

"Condition altered" regulators exhibit altered preference for the set of promoters bound in two different conditions. Ste12 is the best studied of the regulators whose binding behaviour falls into this "altered" class. The specificity of Ste12 is thought to depend, in part, on its interactions with other regulators whose availability is environment-dependent. For example, the binding site preference of Ste12 changes when Tec1 is present, apparently because Tec1 interacts with Ste12 and has its own

DNA-binding site specifity [31,47]. This condition-altered behaviour was also observed for the transcriptional regulators Aft2, Pho4, and Ume6. We postulate that the binding specificity of many of the transcriptional regulators may be altered through interactions with other regulators or through modifications (e.g., chemical) that are environment-dependent.

We note that classification of regulator behaviour is dependent on the environmental conditions selected for comparison, and that some regulators fall into multiple categories. This is consistent with the understanding that multiple types of regulatory mechanisms are often associated with regulators, and differentially modulate regulator behaviour. We anticipate that future experiments in additional environments will provide a more comprehensive understanding of regulator dynamics.

**Challenges for Future Drafts of the Regulatory Code**

We have used extensive *in vivo* binding data, conserved sequence information and prior knowledge of regulator-DNA interactions to construct a first draft of the transcriptional regulatory code of a eukaryote cell. We anticipate that future revisions will be facilitated by collecting more experimental data, by testing models that emerge from the data, and by developing improved computational algorithms to integrate various data types. It will be valuable to collect genome-wide binding data for DNA-binding regulators and chromatin regulators in cells grown under additional environments. Experimental tests of models for regulator functions predicted by their environment-dependent binding behaviour will provide new insights into the regulatory mechanisms involved in control of global gene expression programs. Knowledge of the environment-dependent changes in the abundance, modification state and intracellular compartmentalization of transcriptional regulators will also be valuable, although collecting this evidence will be challenging because transcriptional regulators are

among the least abundant of cellular proteins. Frequent sampling of genome-wide expression data obtained as cells are placed in the new environments will permit investigators to integrate binding and expression data to explain how dynamic changes in gene expression programs are regulated. New computational algorithms will play a key role in improved binding site sequence prediction and allowing integration of various data types to reveal how the transcriptional regulatory code controls gene expression programs under diverse conditions.

## Methods

### Strain Information

Strains were created for each of the 204 regulators in which a repeated Myc epitope coding sequence was integrated into the endogenous gene encoding the regulator. PCR constructs containing the Myc epitope coding sequence and a selectable marker flanked by regions of homology to either the 5' or 3' end of the targeted gene were transformed into the W303 yeast strain Z1256. Genomic integration and expression of the epitope-tagged protein were confirmed by PCR and Western blotting, respectively.

### Genome wide location analysis

Genome-wide location analysis was performed as previously described. Bound proteins were formaldehyde-crosslinked to DNA in vivo, followed by cell lysis and sonication to shear DNA. Crosslinked material was immunoprecipitated with an anti-myc antibody, followed by reversal of the crosslinks to separate DNA from protein. Immunoprecipitated DNA and DNA from an unenriched sample were amplified and differentially fluorescently labeled by ligation-mediated PCR. These samples were

hybridized to a microarrray consisting of spotted PCR products representing the intergenic regions of the *S. cerevisiae* genome. Relative intensities of spots were used as the basis for an error model that assigns a probability score (*P* value) to binding interactions.

**Growth environments**

We profiled all 203 regulators in rich medium. In addition, we profiled 85 regulators in at least one other environmental condition. The list of regulators is given in Supplementary Table S1 and more information about the selection of these regulators and the environmental conditions used can be found at the author's web site.

**Motif discovery**

We used six methods to identify the specific sequences bound by regulators: AlignACE, MEME, MDscan, the method of Kellis et al. and two additional new methods that incorporate conservation data: MEME_c and CONVERGE. MEME_c uses the existing MEME program without change, but applies it to a modified set of sequences in which non-conserved bases were replaced with the letter "N". CONVERGE, is a novel EM-based algorithm that takes a set of multiple sequence alignments (MSA) as input instead of a set of sequences. Each MSA contains the available conservation information for a single probe across the *sensu stricto* species. Rather than searching for sites that are identical across multiple species, as is the case for MEME_c, CONVERGE searches for loci where all aligned sequences are consistent with the same specificity model. CONVERGE is described in greater detail in the supplemental material and will be published elsewhere.

We used XXX statistics to judge the significance of motifs A, B, C ... (described in the supplementary material). To determine the appropriate thresholds for these measures, we applied each program to sets of randomly selected probes and calculated the empirical probability distribution for each program to find a motif with the given score. A motif was accepted if any one of that scores had a p-value $\leq 0.001$ when compared distribution of the same score observed in 50 randomization runs with the same program. Significant motifs for the same factor were clustered and averaged.

**Regulatory code**

Potential binding sites were included in the map of the regulatory code if they satisfied two criteria. First, a locus had to match the specificity model for a regulator in the *Saccharomyces cerevisiae* genome and at least two other *sensu stricto cerevisiae* genomes with a score $\geq$70% of the maximum possible. Second, the locus had to lie in an intergenic region that also contained a probe bound by the corresponding factor ($p \leq 0.001$).

Figure 1. Genome-wide Distribution of Transcriptional Regulators. Genome-wide location analysis was used to determine the genomic occupancy of 203 DNA-binding transcriptional regulators in rich media conditions and, for 85 of these regulators, in at least one of twelve other environmental conditions. Additional information on experimental protocols, the regulators under study, raw location data, and data analysis methods are available in supplementary data and on the author's website[48]. The data selected for further analysis met a 0.001 *P* value threshold (Lee et al., 2002). a. Distribution of the number of promoter regions bound per regulator. For regulators profiled under multiple conditions, the union of promoter regions bound under all conditions is reported (blue). An average of randomized distributions for the same set of *P* values randomly assigned among regulators and promoter regions is shown in pink. b. Pairwise comparison of the number of promoter regions bound under two different conditions for 25 regulators. Dark blue bars represent the number of promoter regions bound under growth in rich medium; light blue bars represent the number of promoter regions bound under growth in amino acid starvation medium.

Figure 2. Binding Site Sequences for Yeast Transcriptional Regulators. a. Experimental procedure. Data from location analysis experiments were subjected to computational analyses to determine regulator binding site sequence specificities. These sequences were compared to and supplemented with published sequence specificities. Phylogenetic conservation information was used both to determine, in part, the binding sequences and in selecting the set of final sequences for inclusion in the map of the regulatory code. b. Specificity models that were "rediscovered" using our location data (left) as well as for newly discovered sequences (right). The height of the letter in each logo

represents the frequency of the given nucleotide at that position within the collection of binding specificities. c. Conservation of bound motifs. The degreee of conservation of genomic sites that match a TRANSFACE matrix and were bound (red) or unbound (dark blue). The distribution of conservation values for all possible six-base pair sequences is shown for comparison (cyan).

Figure 3. Yeast Transcriptional Regulatory Code. A genomic map of regulatory code sequences. Regions of Chromosomes II, IV and VII are shown including the locations of conserved DNA sequences bound *in vivo* by transcriptional regulators. Genes are shown as grey rectangles with arrows indicating direction of transcription; binding sequences are shown as coloured boxes.

Figure 4. Yeast promoter architectures. Four classes of arrangements present in promoter regions are depicted.

Figure 5. Environment-specific Utilization of Transcriptional Regulatory Code. Four patterns of genome-wide binding behaviour are depicted in a graphic representation on the left, where transcriptional regulators are represented by a coloured circle, a representative set of gene/promoters is represented above and below the regulator, and lines between the regulator and the gene/promoters represent binding events. Specific examples of the environment-dependent behaviours are depicted in the middle. Coloured circles represent regulators and coloured boxes represent DNA binding sequences present within promoter regions.

Figures 8-39. Specific Embodiments of the methods described herein.

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**Description of Supplementary Tables and Figures:**

**Supplementary Table S1. List of all factors profiled and conditions used.**

**Supplementary Table S2. List of all binding sequences used for creating map.**

**Supplementary Table S3. Table of Single Regulator MIPs annotations.**

**Supplementary Table S4. List of regulators and genes with repetitive motif architecture.**

**Supplementary Table S5. List of regulators and genes with co-occurring motif architecture.**

**Supplementary Table S6. Classification of all regulators into binding behaviour categories.**

**Supplementary Figure S1. Distance to ATG.**

**Supplementary Figure S2. Gcn4 motifs.**

Supplementary Table S1

| | | |
|---|---|---|
| A1 | Kre33 | Smp1 |
| Abf1 | Kss1 [5,6] | Snf1 |
| Abt1 | Leu3 [3] | Snt2 |
| Aca1 | Mac1 [1] | Sok2 [5] |
| Ace2 | Mal13 | Spt10 |
| Adr1 [3,7] | Mal33 [1,2] | Spt2 |
| Aft2 [1,2] | Mbf1 | Spt23 |
| Arg80 [3] | Mbp1 [1,2] | Srd1 |
| Arg81 [3] | Mcm1 [5,6] | Stb1 |
| Aro80 [3] | Mds3 | Stb2 |
| Arr1 [1] | Met18 | Stb4 |
| Ash1 [5] | Met28 [3] | Stb5 |
| Ask10 | Met31 [3] | Stb6 |
| Azf1 | Met32 [3] | Ste12 [5,6] |
| Bas1 [3] | Met4 [3] | Stp1 [3] |
| Bye1 | Mga1 [1] | Stp2 |
| Cad1 [1,3] | Mig1 [8] | Stp4 |
| Cbf1 [3] | Mig2 [1] | Sum1 |
| Cha4 [3] | Mig3 | Sut1 |
| Cin5 [1,2] | Mot3 [1,2,3] | Sut2 |
| Crz1 | Msn1 | Swi4 |
| Cst6 | Msn2 [1,2,4,7,10] | Swi5 |
| Cup9 | Msn4 [1,2,4,10] | Swi6 |
| Dal80 [4] | Mss11 [5] | Tbs1 |
| Dal81 [3,4] | Mth1 [8] | Tec1 [5,6] |
| Dal82 [3,4] | Ndd1 | Thi2 [12] |
| Dat1 | Ndt80 | Tos8 |
| Dig1 [5,6] | Nnf2 | Tye7 |
| Dot6 | Nrg1 [1,2] | Uga3 [3,4] |
| Ecm22 | Oaf1 | Ume6 [1] |
| Eds1 | Opi1 | Upc2 |
| Fap7 | Pdc2 | Usv1 |
| Fhl1 [1,3,4] | Pdr1 [2] | War1 |
| Fkh1 | Pdr3 | Wtm1 |
| Fkh2 [1,2] | Phd1 [5] | Wtm2 |
| Fzf1 | Pho2 [1,2,3,11] | Xbp1 [2,7] |
| Gal3 | Pho4 [11] | Yap1 [1,2,7] |
| Gal4 [8,9] | Pip2 | Yap3 [1] |
| Gal80 | Ppr1 | Yap5 [1] |
| Gat1 [3,4,7] | Put3 [2,3] | Yap6 [1,2] |
| Gat3 | Rap1 [3] | Yap7 [1,2] |
| Gcn4 [3,4] | Rco1 | YBL054W |
| Gcr1 | Rcs1 [1,2,3] | YBR239C |
| Gcr2 [3] | Rdr1 | YBR267W |

| | | |
|---|---|---|
| Gln3 [3,4] | Rds1 [1] | YDR026C |
| Gts1 | Reb1 [1,2] | YDR049W |
| Gzf3 [1,4] | Rfx1 | YDR266C |
| Haa1 | Rgm1 | YDR520C |
| Hac1 | Rgt1 [8] | YER051W |
| Hal9 | Rim101 [1,2] | YER130C |
| Hap1 | Rlm1 [5] | YER184C |
| Hap2 [4] | Rlr1 | YFL044C |
| Hap3 | Rme1 | YFL052W |
| Hap4 [2,3] | Rox1 [1,2] | YGR067C |
| Hap5 [3] | Rph1 [1,2,3] | Yhp1 |
| Hir1 | Rpi1 | YJL206C [1,2] |
| Hir2 | Rpn4 [1,2] | YKL222C |
| Hir3 | Rtg1 [3,4] | YKR064W |
| Hms1 | Rtg3 [1,2,3,4] | YLR278C |
| Hms2 | Rts2 | YML081W |
| Hog1 | Sfl1 | YNR063W |
| Hsf1 [1,2,7] | Sfp1 [1,2,3] | Yox1 |
| Ifh1 | Sig1 [1] | YPR022C |
| Ime1 [1] | Sip3 | YPR196W |
| Ime4 [1] | Sip4 [3] | Yrr1 |
| Ino2 | Skn7 [1,2,7] | Zap1 |
| Ino4 | Sko1 | Zms1 |
| Ixr1 | Smk1 | |

| | |
|---|---|
| 1 - highly hyperoxic | 7 - elevated temperature |
| 2- moderately hyperoxic | 8 - galactose medium |
| 3 - amino acid starved | 9 - raffinose medium |
| 4 - nutrient deprived | 10 - acidic medium |
| 5 - filamentation inducing | 11 - phosphate deprived medium |
| 6 - mating inducing | 12 - vitamin deprived medium |

Supplementary Table S2

| Regulator | Discovered Specificity[1] | Known Specificity[1,2] | Programs[3] |
|---|---|---|---|
| Abf1 | rTCAyt....Acg | rTCAyT....ACGw | A, C, D, K, M, N |
| Ace2 | tGCTGGT | GCTGGT | K |
| Adr1 | | GGrGk | |
| Aft2 | GGGTGy | "ATCTTCAAAAGTGCACCCAT... ...TTGCAGGTGC" | A, C, D, M, N |
| Arr1 | | TTACTAA | |
| Ash1 | | yTGACT | |
| Azf1 | YwTTkcKkTyyckgykky | AAGAAAAA | N |
| Bas1 | | TGACTC | A, K, M, N |
| Cad1 | mTTAsTmAkC | TTACTAA | A, C, D, M, N |
| Cbf1 | tCACGTG | rTCACrTGA | A, C, D, K, M, N |
| Cin5 | TTAygTAA | TTACTAA | A, C, D |
| Crz1 | | GwGGCTG | |
| Dal80 | GATAA | GATAAG | |
| Dal81 | | AAAAGCCGCGGGCGGGATT | |
| Dal82 | GATAAGa | "GCTGAAAGTTGCGGTGCGATA... ...GAATACCGCGGATTTTGGAA" | K, D |
| Dig1 | TgAAAca | | A, C, D, K, M, N |
| Ecm22 | | CTCGTATAAGC | |
| Fhl1 | rTGTayGGrtg | | A, C, D, K, M, N |
| Fkh1 | tTgTTTac | GGTAAACAA | A, C, D, K, M, N |
| Fkh2 | aaa.GTAAACAa | GGTAAACAA | A, C, D, K, M, N |
| Gal4 | CGG.....cCg | CGG.....CCG | A, K |
| Gal80 | | CGG.....CCG | |
| Gat1 | aGATAAG | GATAA | K |
| Gcn4 | TGAsTCa | ArTGACTCw | A, C, D, K, M, N |
| Gcr1 | GGCTTCCwC | | |
| Gln3 | GATAAGa.a | GATAAGATAAG | C, D, K |
| Gzf3 | GATAAG | GATAA | |
| Hac1 | kGmCAGCGTGTC | | |
| Hap1 | GGmraTA.CGs | CGG...TA.CGG | C, M |
| Hap2 | CCAAT | ayc..ccaat.a.m | |
| Hap3 | CCAAT | ayc..ccaat.a.m | |
| Hap4 | g.CcAAtcA | ayc..ccaat.a.m | A, C, D, M, N |
| Hap5 | CCAAT | | |
| Hsf1 | TTCya.....TTCAGAA | ..TTCTAGAA | A, C, D, K, M, N |
| Ime1 | AAkGAAA.kwA | A | |
| Ino2 | CAcaTGc | GATGTGAAAT | C, D, M, N |
| Ino4 | CATGTGaaaa | CATGTGAAAT | A, C, D, K, M, N |
| Leu3 | cCGgtacCGG | yGCCGGTACCGGyk | A, D, K, M, |
| Mac1 | GAGCAAA | | |
| Mbp1 | rACGCGt | ACGCGT | A, C, D, K, M, N |

| | | | |
|---|---|---|---|
| Mcm1 | tttCC.rAt..gg | wTTCCyAAw..GGTAA | A, C, D, M, N |
| Met31 | AAACTGTGG | | |
| Met32 | AAACTGTGG | | |
| Met4 | RMmAwsTGKSgyGsc | C | |
| Mot3 | yAGGyA | | |
| Msn2 | mAGGGGsgg | mAGGGG | M |
| Msn4 | mAGGGG | | |
| Ndd1 | tt.CC.rAw..GG | A, D | |
| Nrg1 | GGaCCCT | TCCCTCATTTC | A, C, D, M, N |
| Opi1 | TCGAAyC | | |
| Pdr1 | ccGCCgRAwra | CCGCGG | M |
| Pdr3 | TCCGCGGA | | |
| Phd1 | sc.GC.gg | A, D, N | |
| Pho2 | SGTGCGsygyG | N | |
| Pho4 | CACGTGs | cacgtk.g | K, D, N |
| Put3 | CGG.....CCG | | |
| Rap1 | tGyayGGrtg | wrmACCCATACAyy | A, C, D, M, N |
| Rcs1 | ggGTGca.t | AmTGCACCCAkTT | C, D, M, N |
| Rds1 | kCGGCCGa | D, N | |
| Reb1 | CGGGTAA | TTACCCGG | A, C, D, K, M, N |
| Rfx1 | TTgccATggCAAC | D | |
| Rgt1 | CGGA..A | | |
| Rim101 | TGCCAAG | | |
| Rlm1 | CTAwwwwTAG | | |
| Rlr1 | ATTTTCttCwTt | N | |
| Rox1 | ysyATTGTT | | |
| Rph1 | CCCCTTAAGG | AGGGG | |
| Rpn4 | TTTGCCACCGGTGGCAAA | | A, C, D, K, M, N |
| Rtg1 | GGTCAC | | |
| Sfl1 | GAAGCTTC | | |
| Sfp1 | ayCcrtACay | A, C, D, M, N | |
| Sig1 | ArGmAwCrAmAA | M | |
| Sip4 | CGG.y.AATGGrr | yCGGAyrrAwGG | D |
| Skn7 | G.C..GsCs | ATTTGGCyGGsCC | A, C, D, M, N |
| Sko1 | ACGTCA | | |
| Smp1 | ACTACTAwwwwTAG | | |
| Snt2 | yGGCGCTAyca | A, C, D, M, N | |
| Sok2 | tGCAg..a | A | |
| Spt2 | ymtGTmTytAw | M | |
| Spt23 | rAAATsaA | C | |
| Stb1 | rracGCsAaa | C, D, K, M, N | |
| Stb4 | TCGg..CGA | K | |
| Stb5 | CGGwstTAta | CCG | D, N |
| Ste12 | tgAAACa | ATGAAAC | A, C, D, K, M, N |
| Stp1 | rCGGC...rCGGC | | |
| Sum1 | gyGwCAswaaw | AGyGwCACAAAAk | A, C, D, M, N |
| Sut1 | gcsGsg..sG | A, D, M | |
| Swi4 | raCgCsAAA | C.CGAAA | A, C, D, K, M, N |

| | | | |
|---|---|---|---|
| Swi5 | kGCTGr | | |
| Swi6 | tttcGCGt | C.CGAAA | A, C, D, M, N |
| Tec1 | rrGAATG | CATTCy | |
| Thi2 | gmAAcy.twAgA | | C, D |
| Tye7 | tCACGTGAy | CA..TG | A, C, D, M |
| Uga3 | CCG....CGG | | |
| Ume6 | taGCCGCCsa | wGCCGCCGw | A, C, D, K, M, N |
| Xbp1 | CTTCGAG | | |
| Yap1 | TTaGTmAGc | TGAsTCAG | A, C, D, M |
| Yap3 | TTACTAA | | |
| Yap5 | TTACTAA | | |
| Yap6 | TTACTAA | | |
| Yap7 | mTkAsTmAk | TTACTAA | A, C, D, M, N |
| Ydr026C | ttTACCCGGm | | C, D, M, N |
| Yhp1 | TAATTG | | |
| Yox1 | AsAATA.TGAmr | yAATTA | |
| Zap1 | ACCCTmAAGGTyrT | ACCCTAAAGGT | |

1Ambiguity Codes: S = CG, W = AT, R = AG, Y = CT, K = GT, M = AC, "." = ACGT. Letter capitalization reflects the information content at each position of the motif.
2Known specificities are taken from the YPD, SCPD, and TRANSFAC databases.

3Program Codes: A = AlignACE, C = CONVERGE, D = MDscan, K = Kellis et. al, M = MEME, N = MEME (consensus genome)

Supplementary Table OS3

| Regulator | Environment | Functional category |
|---|---|---|
| Cbf1 | SM | amino acid metabolism |
| Dig1 | But | morphogenesis |
| Fhl1 | YPD, SM, Rap, | Protein biosynthesis |
| Fkh1 | YPD | Cell cycle |
| Gal4 | Gal, Raf | carbohydrate metabolism |
| Gcn4 | YPD, SM, Rap | amino acid metabolism |
| Gln3 | YPD | amino acid metabolism |
| Hap1 | YPD | electron transport |
| Hap2 | Rap | cellular respiration |
| Hap4 | YPD | cellular respiration |
| Hsf1 | H2O2Hi, H2O2Lo | response to stress |
| Ino4 | YPD | lipid metabolism |
| Leu3 | YPD | amino acid metabolism |
| Mbp1 | H2O2Hi, H2O2Lo | DNA metabolism |
| Mcm1 | Alpha | Cell cycle |
| Msn2 | H2O2Lo | carbohydrate metabolism |
| Rcs1 | H2O2Hi, H2O2Lo | transport |
| Rds1 | H2O2Hi | carbohydrate metabolism |
| Reb1 | H2O2Lo | vesicle-mediated transport |
| Rpn4 | H2O2Hi, H2O2Lo | protein catabolism |
| Sok2 | But | meiosis |
| Ste12 | YPD, Alpha, But, | conjugation |
| Sum1 | YPD | sporulation |
| Tec1 | But | cell wall organization |
| Yap6 | H2O2Lo | response to stress |

Supplemental Table S4

| Factor | Pvalue | Single | Repetitive | Likely Category | Motif | Bits |
|---|---|---|---|---|---|---|
| SKN7 | 9.85E-01 | O: 41 E: 40 | O: 16 E: 16 | - | G.C..GsCs (1) | 6.0339 |
| MOT3 | 8.42E-01 | O: 6 E: 5 | O: 2 E: 2 | - | yAGGyA (1) | 8.0627 |
| HAC1 | 5.31E-01 | O: 1 E: 0 | O: 0 E: 0 | - | kGmCAGCGTGTC (0) | 18.8864 |
| ROX1 | 1.59E-01 | O: 10 E: 7 | O: 1 E: 3 | - | ysyATTGTT (0) | 12.8397 |
| YAP1 | 1.26E-01 | O: 11 E: 8 | O: 1 E: 3 | - | TTaGTmAGc (1) | 11.5264 |
| RAP1 | 1.48E-01 | O: 34 E: 38 | O: 20 E: 15 | - | tGyayGGrtg (1) | 9.3829 |
| GAL80 | 1.10E-01 | O: 0 E: 0 | O: 1 E: 0 | - | CGG.....CCG (1) | 10.3267 |
| HAP2 | 5.74E-01 | O: 14 E: 12 | O: 4 E: 5 | - | CCAAT (0) | 8.6055 |
| INO2 | 4.77E-02 | O: 11 E: 15 | O: 10 E: 5 | Repetitive | CAcaTGc (1) | 8.8175 |
| HAP1 | 4.74E-03 | O: 31 E: 23 | O: 2 E: 9 | Single | GGmraTA.CGs (1) | 11.2364 |
| HAP4 | 2.10E-01 | O: 20 E: 17 | O: 4 E: 6 | - | g.CcAAtcA (1) | 7.2832 |
| INO4 | 4.90E-01 | O: 15 E: 13 | O: 4 E: 5 | - | CATGTGaaaa (1) | 10.8029 |
| RLM1 | 6.02E-02 | O: 9 E: 6 | O: 0 E: 2 | - | CTAwwwwTAG (0) | 13.6774 |
| MET31 | 8.88E-01 | O: 3 E: 2 | O: 1 E: 1 | - | AAACTGTGG (0) | 15.49 |
| MET32 | 5.65E-01 | O: 8 E: 7 | O: 2 E: 2 | - | AAACTGTGG (0) | 15.49 |
| MCM1 | 4.58E-01 | O: 28 E: 30 | O: 14 E: 11 | - | tttCC.rAt..gg (1) | 7.8147 |
| LEU3 | 6.91E-01 | O: 7 E: 6 | O: 2 E: 2 | - | cCGgtacCGG (1) | 10.3977 |
| SWI6 | 7.36E-06 | O: 34 E: 50 | O: 37 E: 20 | Repetitive | tttcGCGt (1) | 7.33 |
| AZF1 | 5.31E-01 | O: 1 E: 0 | O: 0 E: 0 | - | YwTTkcKkTyyckgykky (1) | 15.7085 |
| RIM101 | 5.31E-01 | O: 1 E: 0 | O: 0 E: 0 | - | TGCCAAG (0) | 12.0478 |
| GAT1 | 8.85E-02 | O: 3 E: 5 | O: 4 E: 1 | - | aGATAAG (1) | 10.571 |
| IME1 | 2.78E-01 | O: 3 E: 2 | O: 0 E: 0 | - | AAkGAAA.kwA (1) | 13.1852 |
| UME6 | 6.31E-03 | O: 55 E: 45 | O: 8 E: 17 | Single | taGCCGCCsa (1) | 10.4956 |
| MBP1 | 2.99E-08 | O: 34 E: 56 | O: 44 E: 21 | Repetitive | rACGCGt (1) | 8.8321 |
| PHO4 | 8.96E-01 | O: 11 E: 10 | O: 4 E: 4 | - | CACGTGs (1) | 8.9549 |
| PHO2 | 2.78E-01 | O: 3 E: 2 | O: 0 E: 0 | - | SGTGCGsygyG (1) | 13.7626 |



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| | | | | | |
|---|---|---|---|---|---|
| STB4 | 6.84E-01 O: 4 E: 3 | O: 1 E: 1 | - | TCGg..CGA (1) | 10.3574 |
| STB5 | 3.75E-01 O: 10 E: 8 | O: 2 E: 3 | - | CGGwstTAta (1) | 10.3136 |
| STB1 | 9.29E-02 O: 6 E: 8 | O: 6 E: 3 | - | rracGCsAaa (1) | 7.7461 |
| NRG1 | 1.63E-01 O: 35 E: 30 | O: 8 E: 12 | - | GGaCCCT (1) | 10.1382 |
| GZF3 | 2.78E-01 O: 3 E: 2 | O: 0 E: 0 | - | GATAAG (0) | 10.3267 |
| SFP1 | 3.03E-02 O: 7 E: 10 | O: 8 E: 4 | Repetitive | ayCcrtACay (1) | 7.8755 |
| YHP1 | 3.76E-01 O: 2 E: 1 | O: 0 E: 0 | - | TAATTG (0) | 10.3267 |
| DIG1 | 1.43E-08 O: 25 E: 45 | O: 38 E: 17 | Repetitive | TgAAAca (1) | 7.5967 |
| MET4 | 1.61E-01 O: 5 E: 3 | O: 0 E: 1 | - | RMmAwsTGKSgyGsc (1) | 16.622 |
| NDD1 | 8.78E-02 O: 21 E: 17 | O: 3 E: 6 | - | tt.CC.rAw..GG (1) | 10.2085 |
| MAC1 | 8.88E-01 O: 3 E: 2 | O: 1 E: 1 | - | GAGCAAA (0) | 12.0478 |
| SNT2 | 8.02E-02 O: 13 E: 10 | O: 1 E: 3 | - | yGGCGCTAyca (1) | 10.9134 |
| ADR1 | 8.98E-01 O: 7 E: 7 | O: 3 E: 2 | - | GGrGk (0) | 6.8387 |
| XBP1 | 3.76E-01 O: 2 E: 1 | O: 0 E: 0 | - | CTTCGAG (0) | 12.0478 |
| ASH1 | 5.31E-01 O: 1 E: 0 | O: 0 E: 0 | - | yTGACT (0) | 9.4432 |
| RPH1 | 5.31E-01 O: 1 E: 0 | O: 0 E: 0 | - | CCCCTTAAGG (0) | 17.2111 |
| PDR3 | 1.10E-01 O: 0 E: 0 | O: 1 E: 0 | - | TCCGCGGA (0) | 13.7689 |
| STE12 | 5.57E-04 O: 48 E: 62 | O: 39 E: 24 | Repetitive | tgAAACa (1) | 7.7973 |
| RCS1 | 4.01E-01 O: 12 E: 13 | O: 7 E: 5 | - | ggGTGca.t (1) | 7.287 |
| TYE7 | 4.24E-01 O: 19 E: 17 | O: 5 E: 6 | - | tCACGTGAy (1) | 10.327 |
| ZAP1 | 3.76E-01 O: 2 E: 1 | O: 0 E: 0 | - | ACCCTmAAGGTyrT (1) | 21.8082 |
| HSF1 | 1.76E-02 O: 49 E: 40 | O: 8 E: 16 | Single | TTCya.....TTC (1) | 8.4321 |
| YDR026c | 4.76E-02 O: 10 E: 7 | O: 0 E: 2 | Single | ttTACCCGGm (1) | 15.1416 |
| SPT2 | 2.01E-01 O: 9 E: 7 | O: 1 E: 2 | - | ymtGTmTytAw (1) | 11.107 |
| SIG1 | 3.76E-01 O: 2 E: 1 | O: 0 E: 0 | - | ArGmAwCrAmAA (1) | 14.1436 |
| SWI4 | 7.29E-04 O: 27 E: 38 | O: 26 E: 14 | Repetitive | raCgCsAAA (1) | 9.5841 |
| GLN3 | 3.01E-01 O: 19 E: 21 | O: 11 E: 8 | - | GATAAGa.a (1) | 9.7858 |



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| | | | | | |
|---|---|---|---|---|---|
| ABF1 | 1.68E-04 O: 119 E: 99 | O: 19 E: 38 | Single | rTCAyt....Acg (1) | 8.7986 |
| PHD1 | 7.89E-03 O: 15 E: 21 | O: 15 E: 8 | Repetitive | sc.GC.gg (1) | 6.6163 |
| STP1 | 5.31E-01 O: 1 E: 0 | O: 0 E: 0 | - | rCGGC...rCGGC (1) | 15.4442 |
| SUM1 | 3.56E-01 O: 22 E: 24 | O: 12 E: 9 | - | gyGwCAswaaw (1) | 7.5512 |
| RFX1 | 4.14E-01 O: 6 E: 5 | O: 1 E: 1 | - | TTgccATggCAAC (1) | 14.949 |
| AFT2 | 9.73E-03 O: 22 E: 29 | O: 19 E: 11 | Repetitive | GGGTGy (1) | 6.2628 |
| RGT1 | 5.69E-03 O: 0 E: 2 | O: 3 E: 0 | - | CGGA..A (1) | 8.6055 |
| PDR1 | 3.76E-01 O: 2 E: 1 | O: 0 E: 0 | - | ccGCCgRAwra (1) | 11.0187 |
| YAP3 | 5.31E-01 O: 1 E: 0 | O: 0 E: 0 | - | TTACTAA (0) | 12.0478 |
| YAP5 | 5.31E-01 O: 1 E: 0 | O: 0 E: 0 | - | TTACTAA (0) | 12.0478 |
| YAP7 | 4.74E-02 O: 43 E: 36 | O: 8 E: 14 | Single | mTkAsTmAk (1) | 8.554 |
| YAP6 | 2.78E-01 O: 3 E: 2 | O: 0 E: 0 | - | TTACTAA (0) | 12.0478 |
| GCN4 | 9.62E-01 O: 67 E: 66 | O: 26 E: 26 | - | TGAsTCa (1) | 7.7817 |
| OPI1 | 3.76E-01 O: 2 E: 1 | O: 0 E: 0 | - | TCGAAyC (0) | 11.1643 |
| TEC1 | 5.02E-02 O: 18 E: 22 | O: 14 E: 9 | - | rrGAATG (1) | 8.4134 |
| YOX1 | 2.10E-01 O: 4 E: 2 | O: 0 E: 1 | - | AsAATA.TGAmr (1) | 16.2819 |
| GCR1 | 3.32E-01 O: 2 E: 2 | O: 2 E: 1 | - | GGCTTCCwC (0) | 14.6065 |
| CAD1 | 6.02E-02 O: 9 E: 6 | O: 0 E: 2 | - | mTTAsTmAkC (1) | 12.0785 |
| RLR1 | 2.10E-01 O: 4 E: 2 | O: 0 E: 1 | - | ATTTTCttCwTt (1) | 13.9257 |
| CBF1 | 2.49E-03 O: 87 E: 73 | O: 15 E: 28 | Single | tCACGTG (1) | 10.7486 |
| FKH2 | 4.13E-01 O: 40 E: 37 | O: 12 E: 14 | - | aaa.GTAAACAa (1) | 10.6377 |
| SKO1 | 4.14E-01 O: 6 E: 5 | O: 1 E: 1 | - | ACGTCA (0) | 10.3267 |
| HAP3 | 3.75E-01 O: 10 E: 8 | O: 2 E: 3 | - | CCAAT (0) | 8.6055 |
| FKH1 | 6.66E-01 O: 43 E: 44 | O: 19 E: 17 | - | tTgTTTac (1) | 8.864 |
| HAP5 | 6.70E-01 O: 13 E: 12 | O: 4 E: 4 | - | CCAAT (0) | 8.6055 |
| RDS1 | 4.14E-01 O: 6 E: 5 | O: 1 E: 1 | - | kCGGCCGa (1) | 10.3555 |
| MSN4 | 5.74E-01 O: 14 E: 12 | O: 4 E: 5 | - | mAGGGG (0) | 9.4432 |

| | | | | | |
|---|---|---|---|---|---|
| FHL1 | 5.26E-02 O: 37 E: 43 | O: 24 E: 17 | - | rTGTayGGrtg (1) | 10.6834 |
| SIP4 | 1.38E-01 O: 1 E: 2 | O: 2 E: 0 | - | CGG.y.AATGGrr (1) | 13.221 |
| ARR1 | 5.31E-01 O: 1 E: 0 | O: 0 E: 0 | - | TTACTAA (0) | 12.0478 |
| BAS1 | 2.84E-04 O: 6 E: 12 | O: 12 E: 5 | Repetitive | TGACTC (1) | 10.093 |
| REB1 | 1.24E-05 O: 135 E: 110 | O: 19 E: 43 | Single | CGGGTAA (1) | 9.5418 |
| SWI5 | 1.05E-02 O: 11 E: 16 | O: 12 E: 6 | Repetitive | kGCTGr (0) | 8.5598 |
| GAL4 | 6.91E-01 O: 7 E: 6 | O: 2 E: 2 | - | CGG.....cCg (1) | 6.7079 |
| DAL81 | 5.31E-01 O: 1 E: 0 | O: 0 E: 0 | - | AAAAGCCGCGGGCGGGATT (0) | 32.7011 |
| DAL80 | 3.24E-01 O: 7 E: 5 | O: 1 E: 2 | - | GATAA (0) | 8.6055 |
| DAL82 | 9.45E-02 O: 11 E: 14 | O: 9 E: 5 | - | GATAAGa (1) | 9.8108 |
| CIN5 | 3.31E-01 O: 42 E: 38 | O: 12 E: 15 | - | TTAygTAA (1) | 8.7034 |
| RTG3 | 1.61E-01 O: 5 E: 3 | O: 0 E: 1 | - | GGTCAC (0) | 10.3267 |
| SMP1 | 3.76E-01 O: 2 E: 1 | O: 0 E: 0 | - | ACTACTAwwwwTAG (0) | 20.5618 |
| SOK2 | 1.34E-05 O: 13 E: 24 | O: 21 E: 9 | Repetitive | tGCAg..a (1) | 6.2038 |
| UGA3 | 5.31E-01 O: 1 E: 0 | O: 0 E: 0 | - | CCG....CGG (1) | 10.3267 |
| SPT23 | 2.30E-01 O: 16 E: 13 | O: 3 E: 5 | - | rAAATsaA (1) | 9.171 |
| THI2 | 1.25E-01 O: 6 E: 4 | O: 0 E: 1 | - | gmAAcy.twAgA (1) | 9.9758 |
| PUT3 | 8.43E-01 O: 2 E: 2 | O: 1 E: 0 | - | CGG.....CCG (1) | 10.3267 |
| SUT1 | 7.85E-01 O: 11 E: 11 | O: 5 E: 4 | - | gcsGsg..sG (1) | 6.5317 |
| RPN4 | 4.01E-04 O: 43 E: 32 | O: 2 E: 12 | Single | TTTGCCACC (1) | 13.3079 |
| ACE2 | 8.85E-02 O: 3 E: 5 | O: 4 E: 1 | - | tGCTGGT (1) | 10.4997 |
| MSN2 | 1.53E-02 O: 15 E: 10 | O: 0 E: 4 | Single | mAGGGGsgg (1) | 11.0666 |



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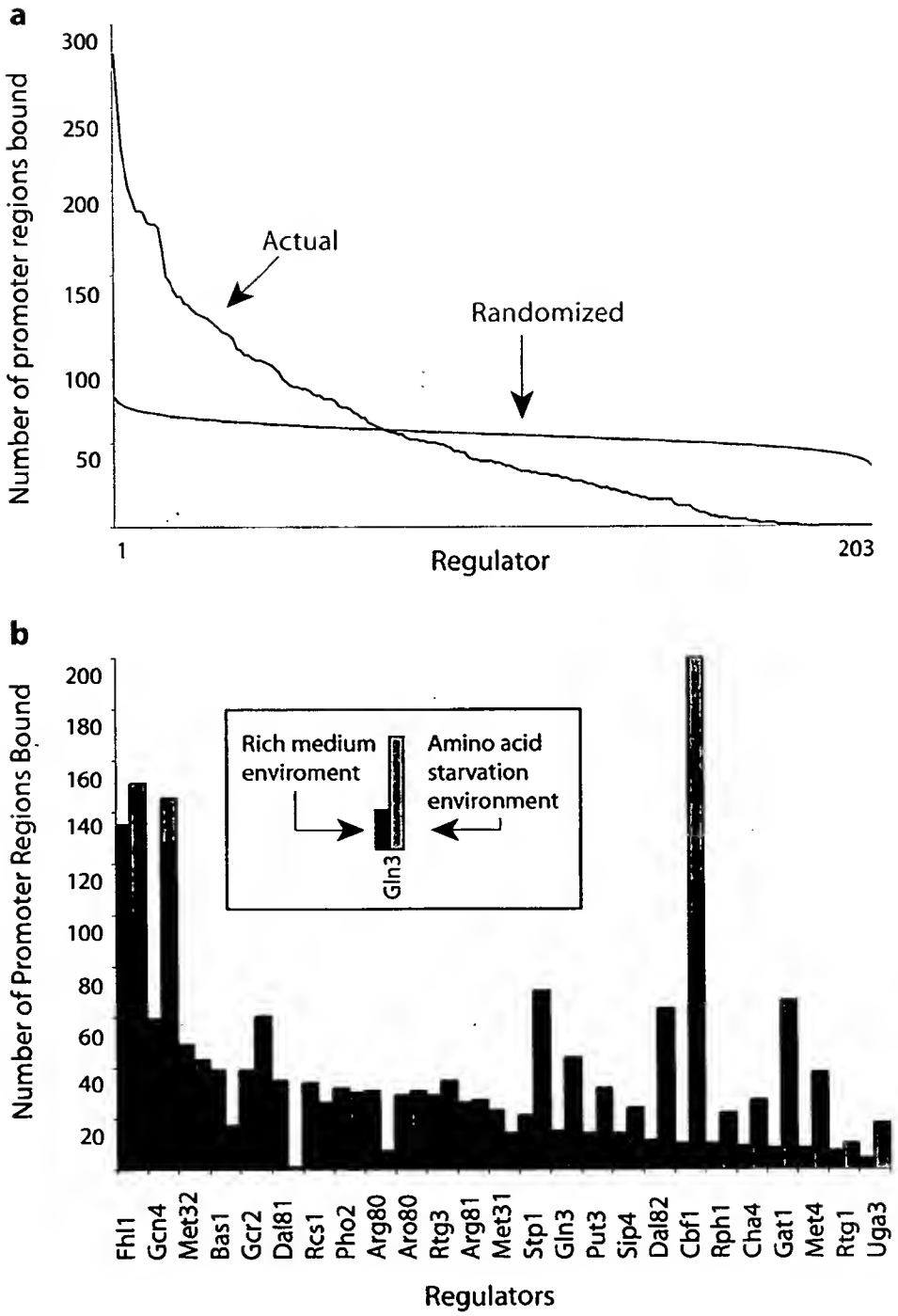
Supplementary Table S5

| Regulator 1 | Regulator 2 | P value | Bound by Regulator 1 | Bound by Regulator 2 | Bound by both |
|---|---|---|---|---|---|
| ACE2 | SWI5 | 0 | 11 | 23 | 5 |
| AFT2 | RCS1 | 0 | 41 | 19 | 11 |
| ARR1 | YAP3 | 0.001 | 1 | 1 | 1 |
| AZF1 | GZF3 | 0.002 | 1 | 3 | 1 |
| BAS1 | MET4 | 0.002 | 18 | 5 | 2 |
| CAD1 | YAP1 | 0 | 9 | 12 | 4 |
| CAD1 | YAP7 | 0 | 9 | 51 | 9 |
| CBF1 | MET31 | 0.002 | 102 | 4 | 3 |
| CBF1 | MET32 | 0 | 102 | 10 | 6 |
| CBF1 | MET4 | 0.004 | 102 | 5 | 3 |
| CBF1 | PHO4 | 0.001 | 102 | 15 | 6 |
| CBF1 | TYE7 | 0 | 102 | 24 | 17 |
| CIN5 | PHD1 | 0 | 54 | 30 | 8 |
| CIN5 | SKN7 | 0 | 54 | 57 | 9 |
| CIN5 | SOK2 | 0 | 54 | 34 | 9 |
| CIN5 | SUT1 | 0 | 54 | 26 | 6 |
| CIN5 | XBP1 | 0.002 | 54 | 2 | 2 |
| CIN5 | YAP6 | 0.005 | 54 | 3 | 2 |
| DAL82 | GAT1 | 0 | 20 | 7 | 3 |
| DAL82 | GLN3 | 0 | 20 | 30 | 7 |
| DAL82 | HAP2 | 0.002 | 20 | 18 | 3 |
| DIG1 | MCM1 | 0 | 63 | 42 | 9 |
| DIG1 | STE12 | 0 | 63 | 87 | 49 |
| DIG1 | SWI4 | 0 | 63 | 53 | 14 |
| DIG1 | SWI6 | 0 | 63 | 71 | 12 |
| DIG1 | TEC1 | 0 | 63 | 32 | 16 |

| | | | | | |
|---|---|---|---|---|---|
| FHL1 | RAP1 | 0 | 61 | 54 | 30 |
| FHL1 | SFP1 | 0 | 61 | 15 | 13 |
| FKH1 | FKH2 | 0 | 62 | 52 | 20 |
| FKH2 | MCM1 | 0 | 52 | 42 | 12 |
| FKH2 | NDD1 | 0 | 52 | 24 | 14 |
| FKH2 | SWI6 | 0 | 52 | 71 | 12 |
| GCN4 | GLN3 | 0.004 | 93 | 30 | 7 |
| GCN4 | LEU3 | 0.002 | 93 | 9 | 4 |
| GCR1 | TYE7 | 0.002 | 4 | 24 | 2 |
| GLN3 | HAP2 | 0 | 30 | 18 | 5 |
| GZF3 | PDR1 | 0.005 | 3 | 2 | 1 |
| HAP2 | HAP3 | 0 | 18 | 12 | 7 |
| HAP2 | HAP4 | 0 | 18 | 24 | 5 |
| HAP2 | HAP5 | 0 | 18 | 17 | 10 |
| HAP3 | HAP5 | 0 | 12 | 17 | 6 |
| HAP4 | HAP5 | 0 | 24 | 17 | 4 |
| HSF1 | MSN4 | 0.001 | 57 | 18 | 5 |
| INO2 | INO4 | 0 | 21 | 19 | 9 |
| INO4 | SKO1 | 0.004 | 19 | 7 | 2 |
| MAC1 | RCS1 | 0.001 | 4 | 19 | 2 |
| MAC1 | SUT1 | 0.002 | 4 | 26 | 2 |
| MBP1 | STB1 | 0 | 78 | 12 | 8 |
| MBP1 | SWI4 | 0 | 78 | 53 | 28 |
| MBP1 | SWI6 | 0 | 78 | 71 | 36 |
| MCM1 | NDD1 | 0 | 42 | 24 | 15 |
| MCM1 | STE12 | 0.001 | 42 | 87 | 9 |
| MCM1 | SWI4 | 0.001 | 42 | 53 | 7 |
| MCM1 | SWI6 | 0 | 42 | 71 | 9 |
| MCM1 | TEC1 | 0.003 | 42 | 32 | 5 |

| | | | | | |
|---|---|---|---|---|---|
| MET31 | MET32 | 0 | 4 | 10 | 4 |
| MET31 | MET4 | 0 | 4 | 5 | 4 |
| MET32 | MET4 | 0 | 10 | 5 | 4 |
| MOT3 | ROX1 | 0.002 | 8 | 11 | 2 |
| MOT3 | SKN7 | 0.004 | 8 | 57 | 3 |
| MSN2 | MSN4 | 0 | 15 | 18 | 4 |
| MSN4 | NRG1 | 0.002 | 18 | 43 | 4 |
| NRG1 | RLM1 | 0.002 | 43 | 9 | 3 |
| NRG1 | SKN7 | 0 | 43 | 57 | 9 |
| NRG1 | SUT1 | 0.001 | 43 | 26 | 5 |
| OPI1 | PDC2 | 0 | 2 | 2 | 2 |
| OPI1 | YML081W | 0 | 2 | 2 | 2 |
| PDC2 | YML081W | 0 | 2 | 2 | 2 |
| PHD1 | ROX1 | 0 | 30 | 11 | 4 |
| PHD1 | SKN7 | 0 | 30 | 57 | 12 |
| PHD1 | SOK2 | 0 | 30 | 34 | 15 |
| PHD1 | SUT1 | 0 | 30 | 26 | 11 |
| PHD1 | SWI6 | 0 | 30 | 71 | 8 |
| RAP1 | SFP1 | 0 | 54 | 15 | 9 |
| RIM101 | YOX1 | 0.003 | 1 | 4 | 1 |
| RLM1 | SKO1 | 0.001 | 9 | 7 | 2 |
| RLR1 | SPT2 | 0 | 12 | 10 | 4 |
| ROX1 | SUT1 | 0 | 11 | 26 | 4 |
| SIP4 | STP1 | 0.002 | 3 | 1 | 1 |
| SKN7 | SOK2 | 0 | 57 | 34 | 12 |
| SKN7 | SUT1 | 0 | 57 | 26 | 10 |
| SKN7 | SWI6 | 0 | 57 | 71 | 11 |
| SKN7 | XBP1 | 0.002 | 57 | 2 | 2 |
| SKO1 | SOK2 | 0.001 | 7 | 34 | 3 |

| | | | | | |
|---|---|---|---|---|---|
| SOK2 | SUT1 | 0 | 34 | 26 | 10 |
| SOK2 | SWI6 | 0.002 | 34 | 71 | 7 |
| STB1 | SWI4 | 0 | 12 | 53 | 10 |
| STB1 | SWI6 | 0 | 12 | 71 | 10 |
| STB1 | TEC1 | 0.003 | 12 | 32 | 3 |
| STE12 | SWI4 | 0 | 87 | 53 | 15 |
| STE12 | SWI6 | 0 | 87 | 71 | 16 |
| STE12 | TEC1 | 0 | 87 | 32 | 18 |
| SWI4 | SWI6 | 0 | 53 | 71 | 43 |
| SWI4 | TEC1 | 0 | 53 | 32 | 11 |
| SWI6 | TEC1 | 0 | 71 | 32 | 11 |
| YAP1 | YAP7 | 0 | 12 | 51 | 8 |
| YAP6 | YAP7 | 0.004 | 3 | 51 | 2 |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250
200
150
100
50
Actual
Randomized
1
Regulator
203
b
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180
160
140
120
100
80
60
40
20
Rich medium enviroment
Amino acid starvation environment
Gln3
Fhl1
Gcn4
Met32
Bas1
Gcr2
Dal81
Rcs1
Pho2
Arg80
Aro80
Rtg3
Arg81
Met31
Stp1
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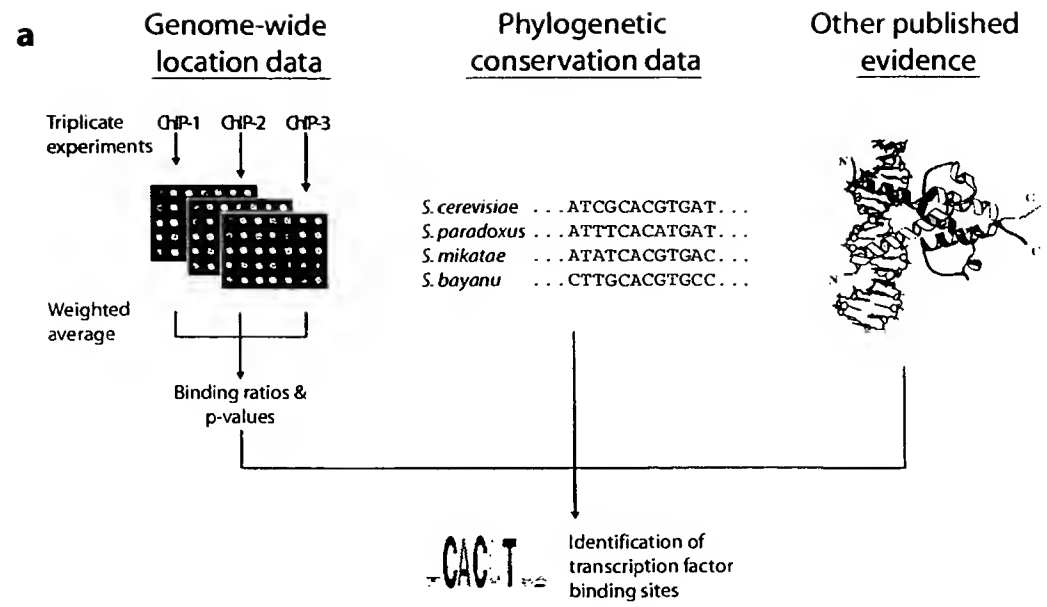
Cbf1
Rph1
Cha4
Gat1
Met4
Rtg1
Uga3
Regulators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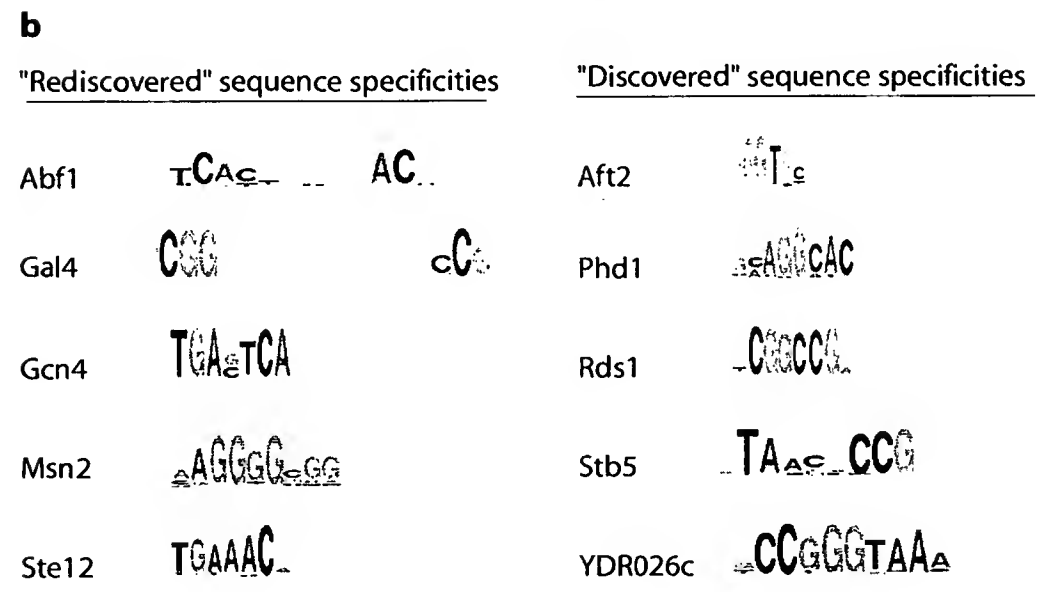
Genome-wide location data
Phylogenetic conservation data
Other published evidence
Triplicate experiments
ChIP-1
ChIP-2
ChIP-3
Weighted average
Binding ratios & p-values
S. cerevisiae . . . ATCGCACGTGAT. . .
S. paradoxus . . . ATTTCACATGAT. . .
S. mikatae . . . ATATCACGTGAC. . .
S. bayanu . . . CTTGCACGTGCC. . .
Identification of transcription factor binding sites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"Rediscovered" sequence specificities
"Discovered" sequence specificities
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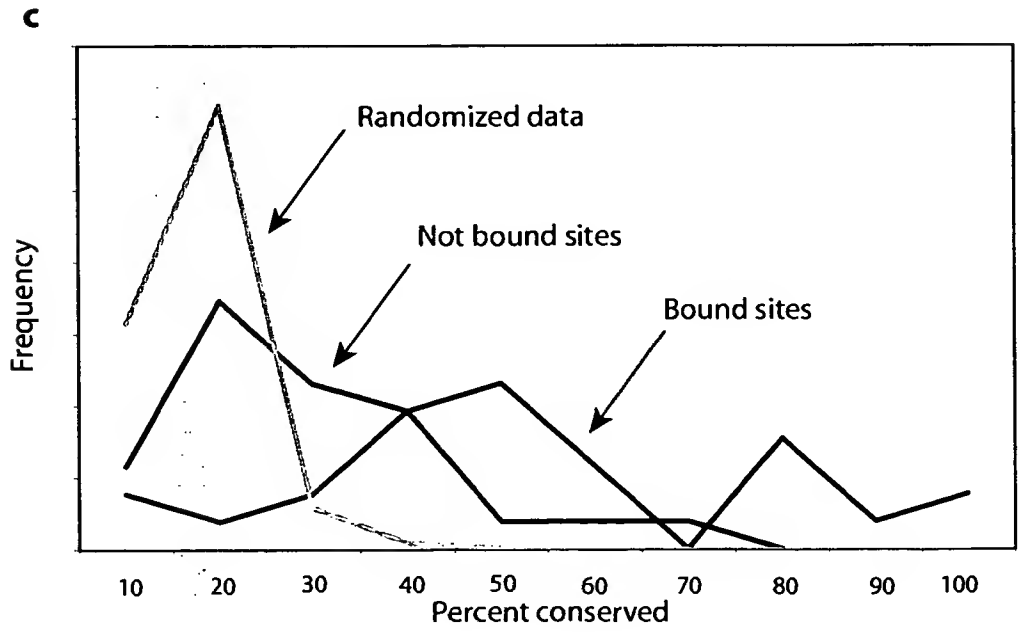
Gcn4
Msn2
Ste12
Aft2
Phd1
Rds1
Stb5
YDR026c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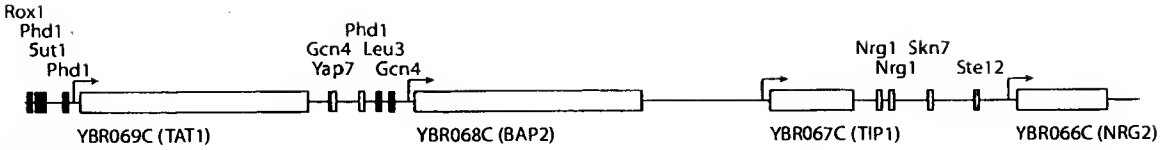
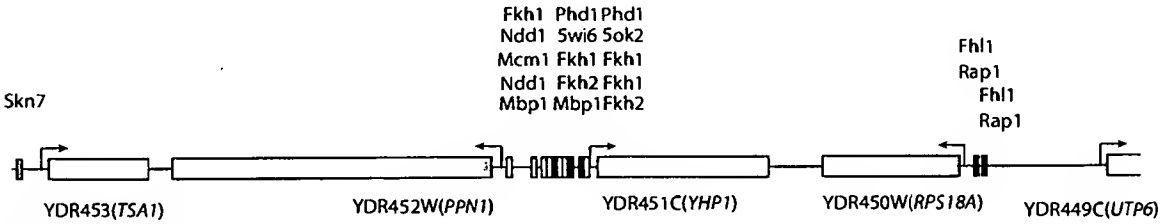
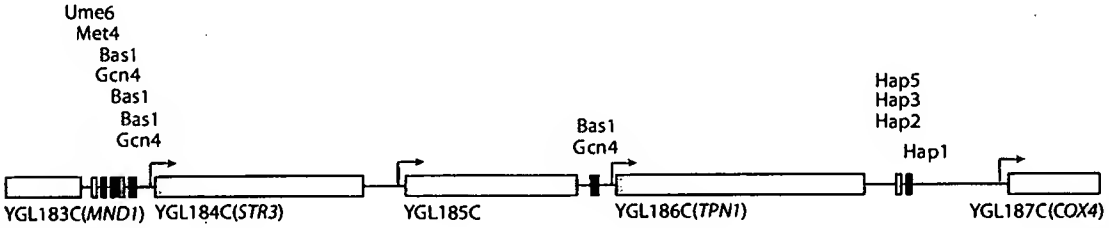
Randomized data
Not bound sites
Bound sites
Frequency
10
20
30
40
50
60
70
80
90
100
Percent conserved




Chromosome II
(370000:379000)



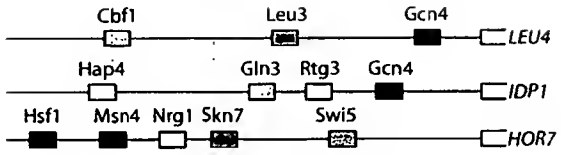

Chromosome IV
(1358800:1366600)



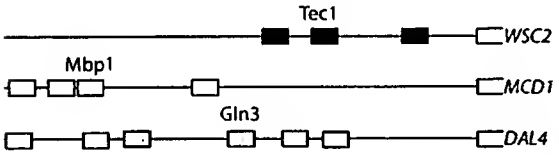

Chromosome V
(150000:157000)

Single regulator

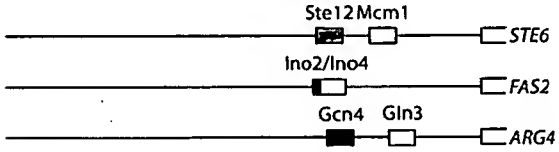

Multiple regulators




Repetitive motifs



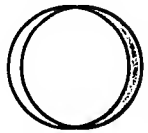

Co-occurring regulators



Global Behavior

Selected Regulator-Gene Interactions

Condition Invariant (e.g. Leu3)



Environment 1: Leu3 → *LEU4*; Leu3 → *ILV2*; Leu3 → *ILV5*

Environment 2: Leu3 → *LEU4*; Leu3 → *ILV2*; Leu3 → *ILV5*

Condition Enabled (e.g. Msn2)

Environment 1: *GSY2*; *CCP1*; *GPH1*

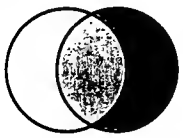
Environment 2: Msn2 → *GSY2*; Msn2 → *CCP1*; Msn2 → *GPH1*

Condition Expanded (e.g. Gcn4)

Environment 1: *ASN1*; Gcn4 → *ARG8*; *BNA1*

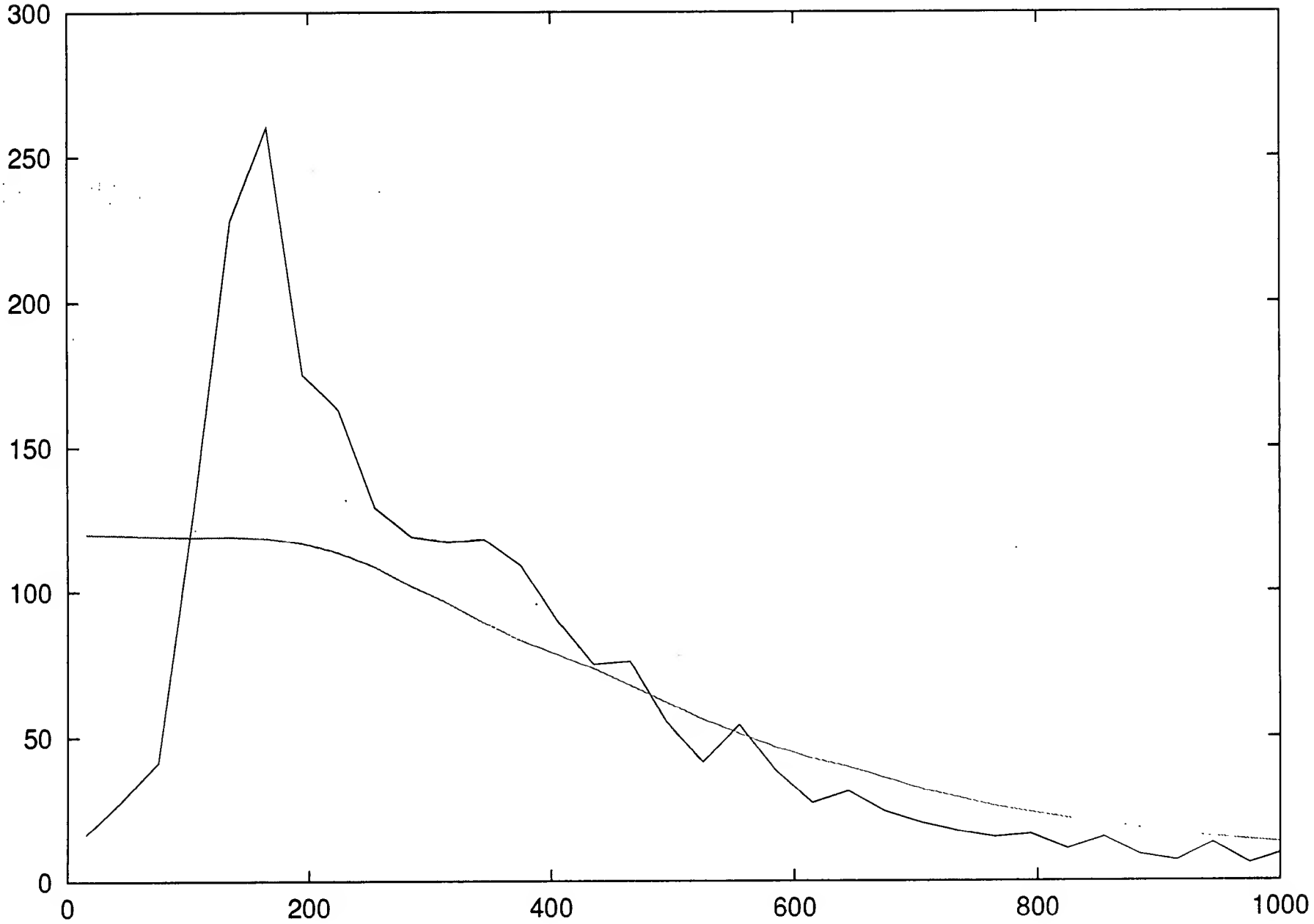
Environment 2: Gcn4 → *ASN1*; Gcn4 → *ARG8*; Gcn4 → *BNA1*

Condition Altered (e.g. Ste12)

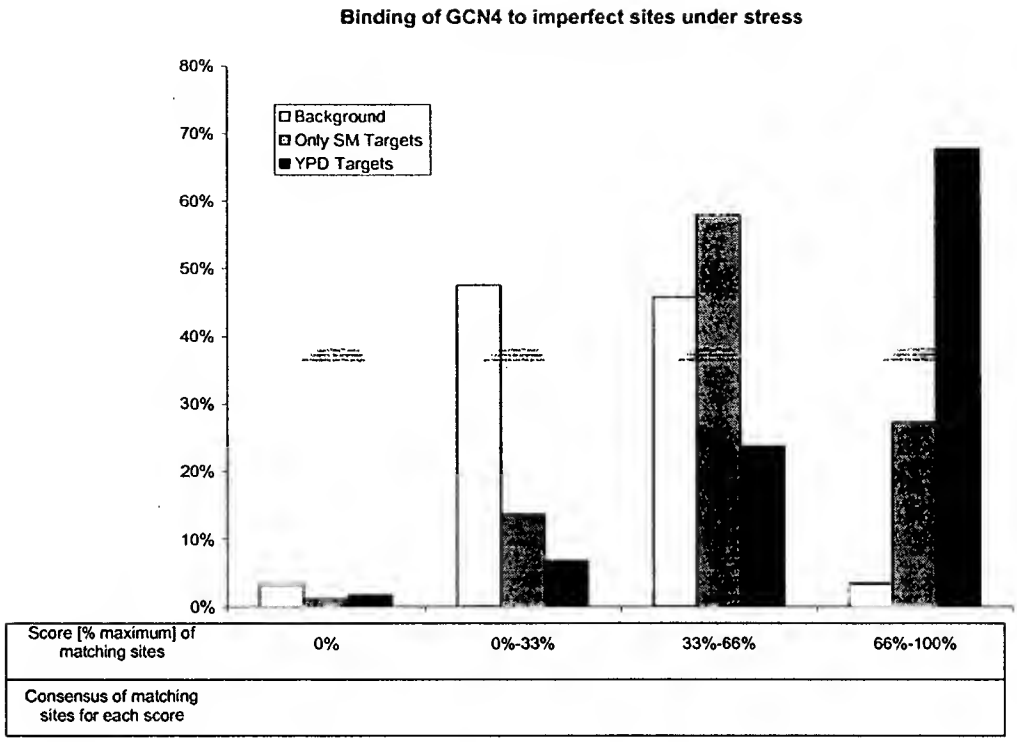


Environment 1: Ste12 → *AGA1*; Ste12 → *STE12*; *MSB2*

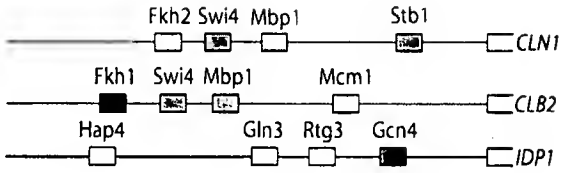
Environment 2: *AGA1*; Ste12 → *STE12*; Ste12 → *MSB2*


300
250
200
150
100
50
0
0
200
400
600
800
1000

Supplemenary Figure S2



# A Draft Transcriptional Regulatory Code for Yeast




- Introduction
  - Concept
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  - DNA sequence specificities
- Promoter Architectures
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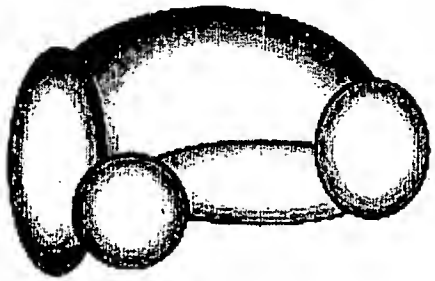
# Regulation of Gene Expression

DNA-binding Regulators

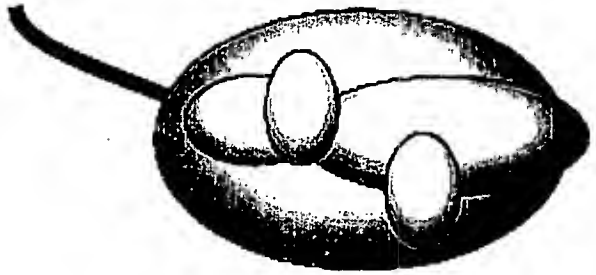
Recognize specific sequences

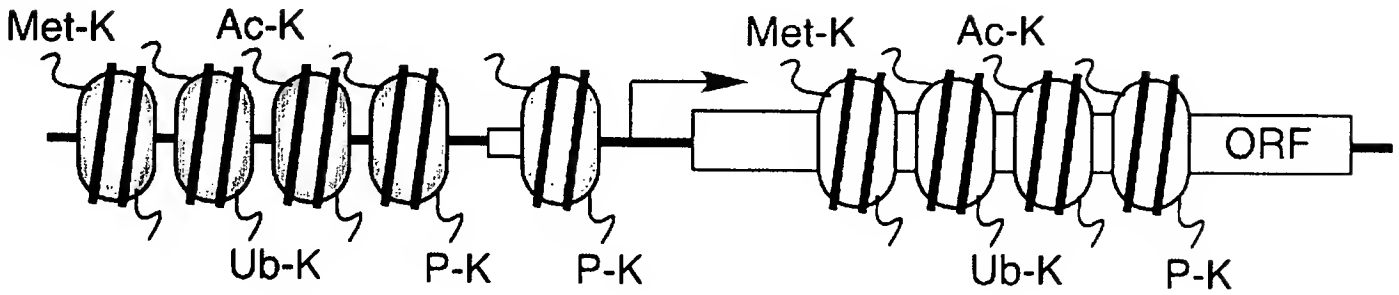
Chromatin Regulators



Control access to sequence

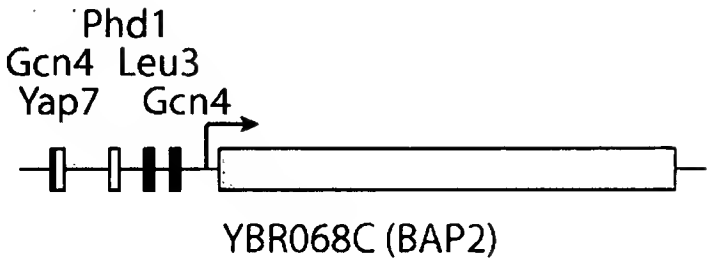
Transcription Initiation Apparatus






We have considerable knowledge of molecular mechanisms that operate at individual genes, but limited knowledge of global regulatory mechanisms.

# Transcriptional Regulatory Code Information




What regulators contribute to control of each gene?

What sequences do they bind?

When do the regulators bind these sequences?

# Previous Methods for Identifying Regulatory Regions

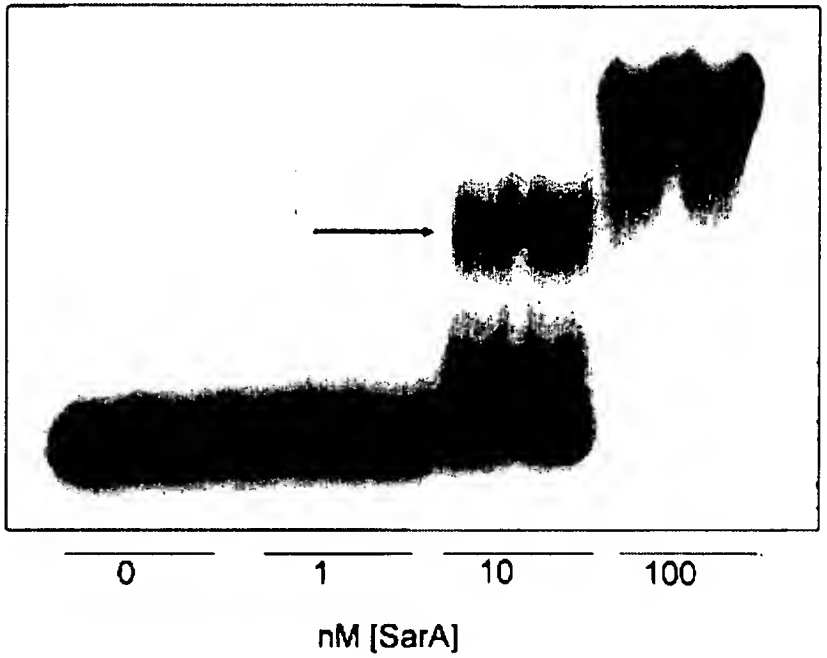
## Genetic

| Genotype | Beta-Galactosidase | |
|---|---|---|
| | Noninduced | Induced |
| $O^+ Z^+ Y^+$ | - | + |
| $O^+ Z^+ Y^+ / F O^+ Z^- Y^+$ | - | + |
| $O^c Z^+ Y^+$ | + | + |
| $O^+ Z^+ Y^- / F O^c Z^+ Y^+$ | + | + |
| $O^+ Z^+ Y^+ / F O^c Z^- Y^+$ | - | + |
| $O^+ Z^- Y^+ / F O^c Z^+ Y^-$ | + | + |

# Previous Methods for Identifying Regulatory Regions

Genetic

Biochemical



# Previous Methods for Identifying Regulatory Regions

Genetic

Biochemical

Alignment

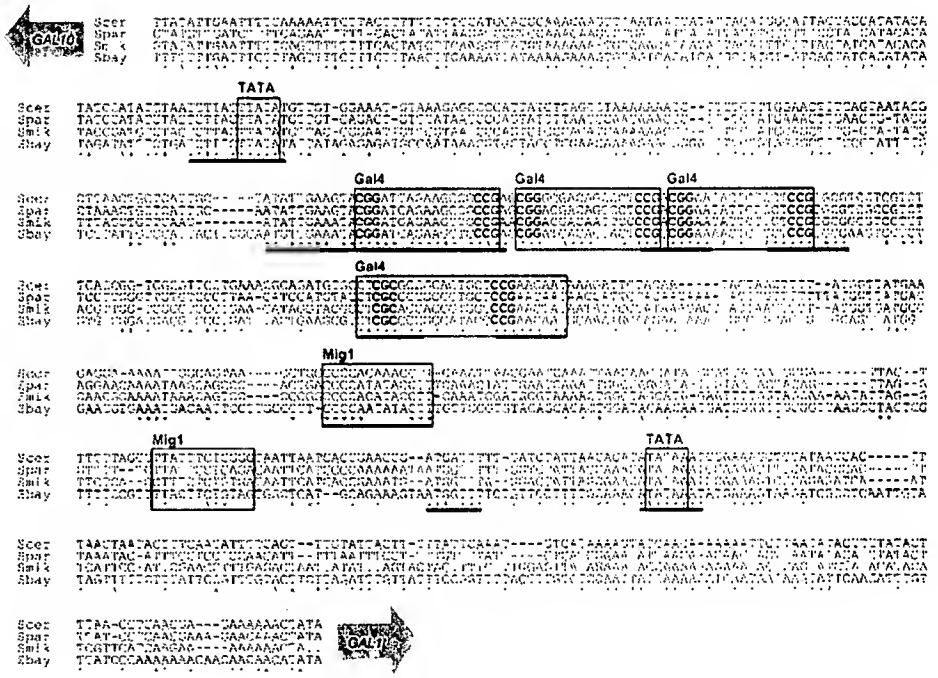
# Previous Methods for Identifying Regulatory Regions

Genetic

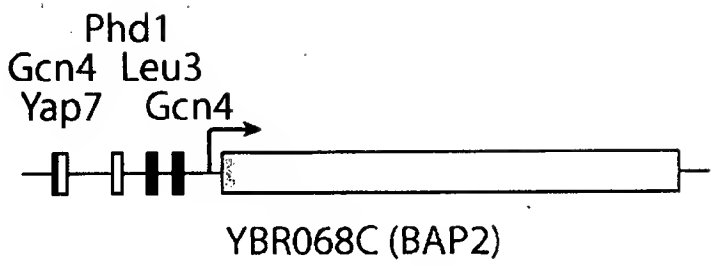
Biochemical

Alignment

Comparative Genomics



# Transcriptional Regulatory Code Information

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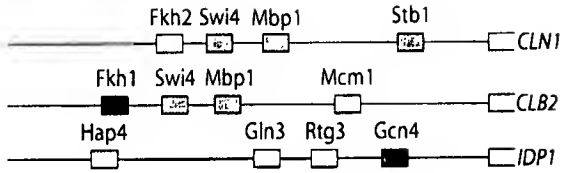
# A Draft Transcriptional Regulatory Code for Yeast

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- DNA sequence specificities

## Promoter Architectures

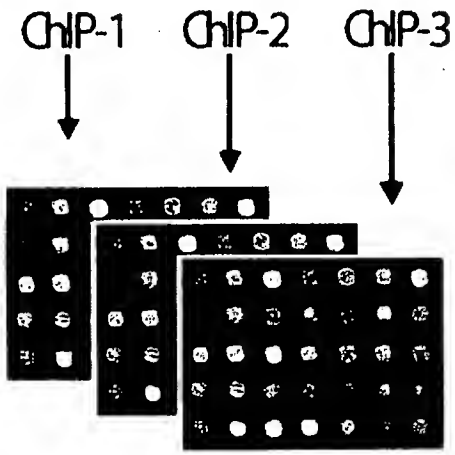
## Environment-dependent Use of Code

# Combining Information to Discover Regulatory Code

Phylogenetic conservation data

*S. cerevisiae* ...ATCGCACGTGAT...
*S. paradoxus* ...ATTTCACATGAT...
*S. mikatae* ...ATATCACGTGAC...
*S. bayanu* ...CTTGCACGTGCC...

Genome wide binding data



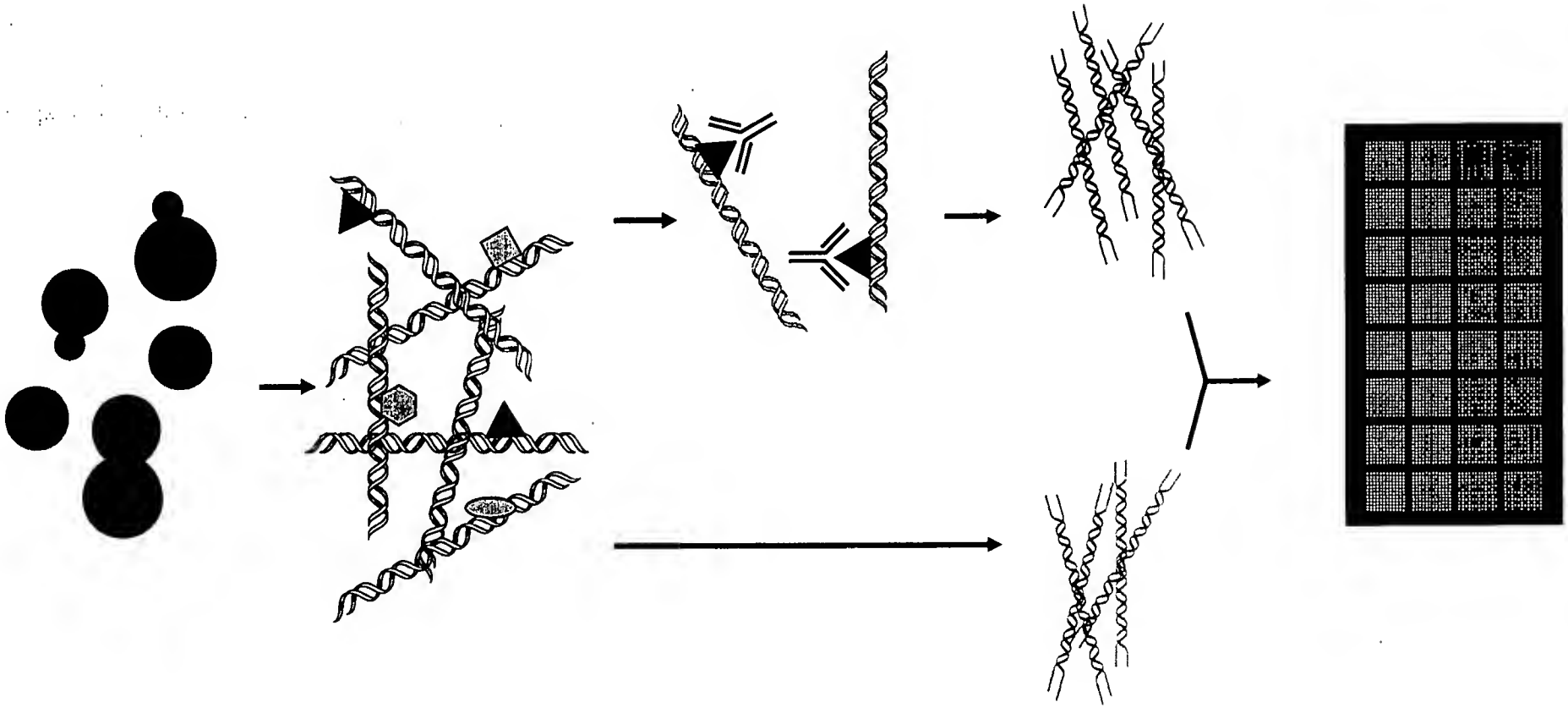

Weighted average:
Binding ratios and
P values

Other published evidence



Identification of
transcription factor
binding sites

# Genome-wide Location Analysis



Crosslink protein to binding sites in living cells

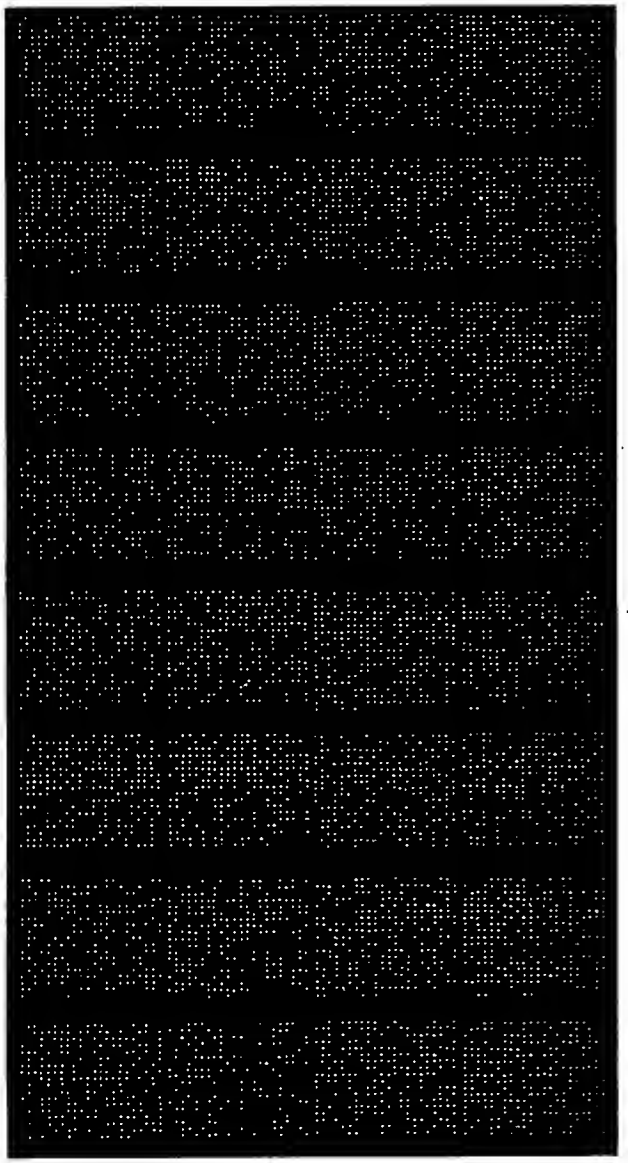
Harvest cells and fragment DNA

Enrich for protein-bound DNA fragments with antibodies

Differentially label the two pools of DNA

Hybridize to microarray and compare ratios of intensities

# Design and Manufacture of Intergenic Arrays



## Content

Probe selection
- Intergenic regions

Coverage
- ~6300 IGR spots
- 95% of intergenic regions

Probe generation
- PCR products
- 95% success rate

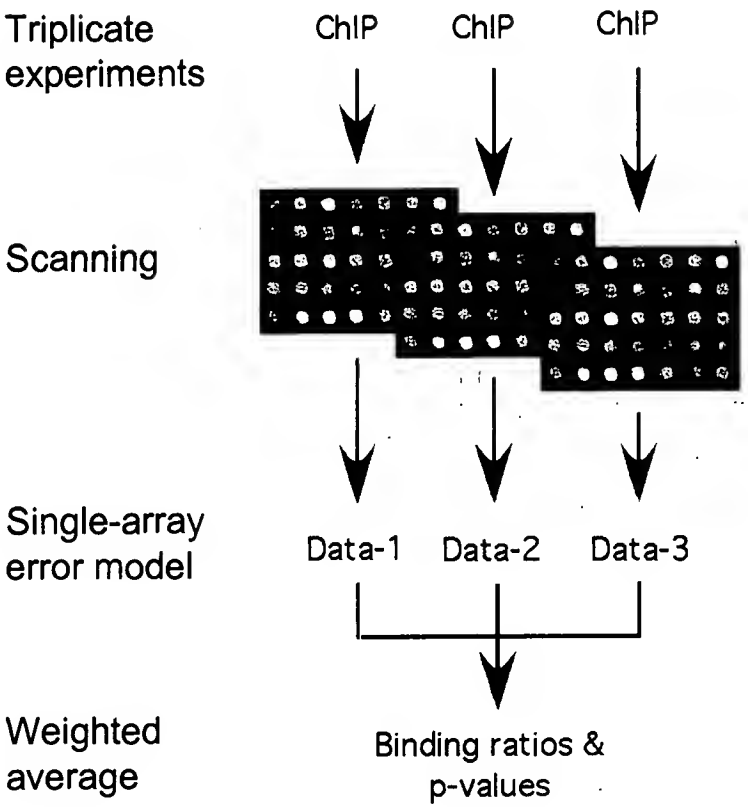
Controls
- Internal ORF regions
- Subgrid controls
- Serial dilution controls
- Exogenous DNA

## Attachment Chemistry

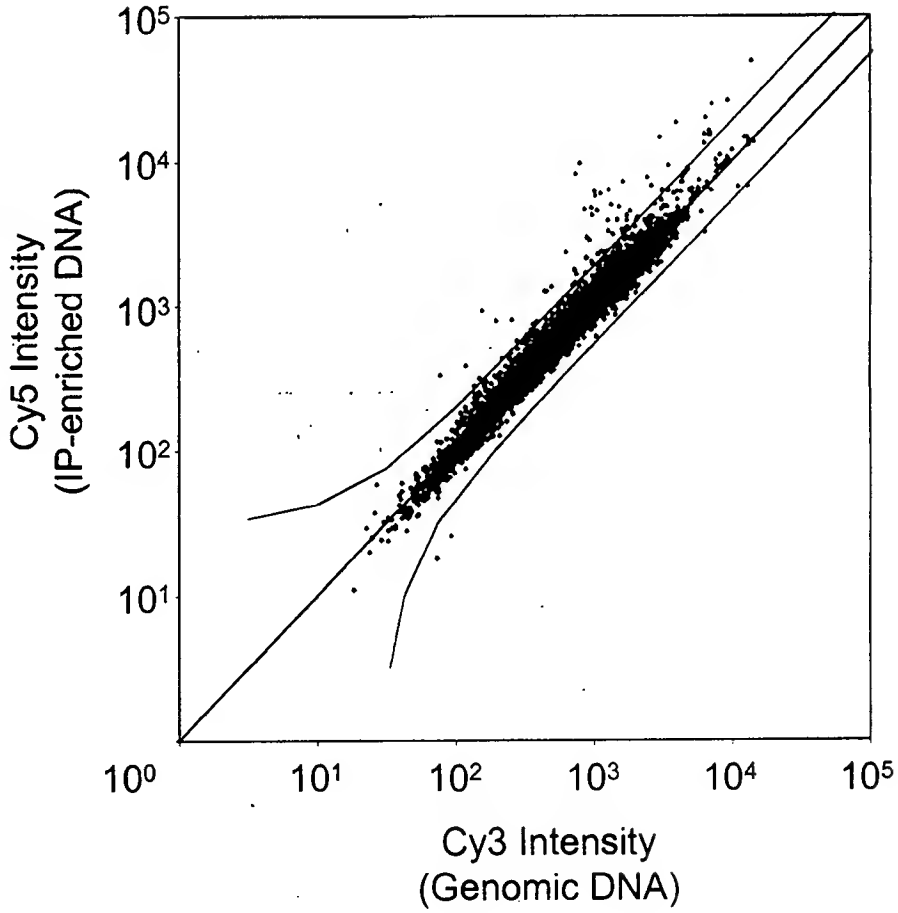
- Aminosilane

# Analysis of Genome-wide Location Data

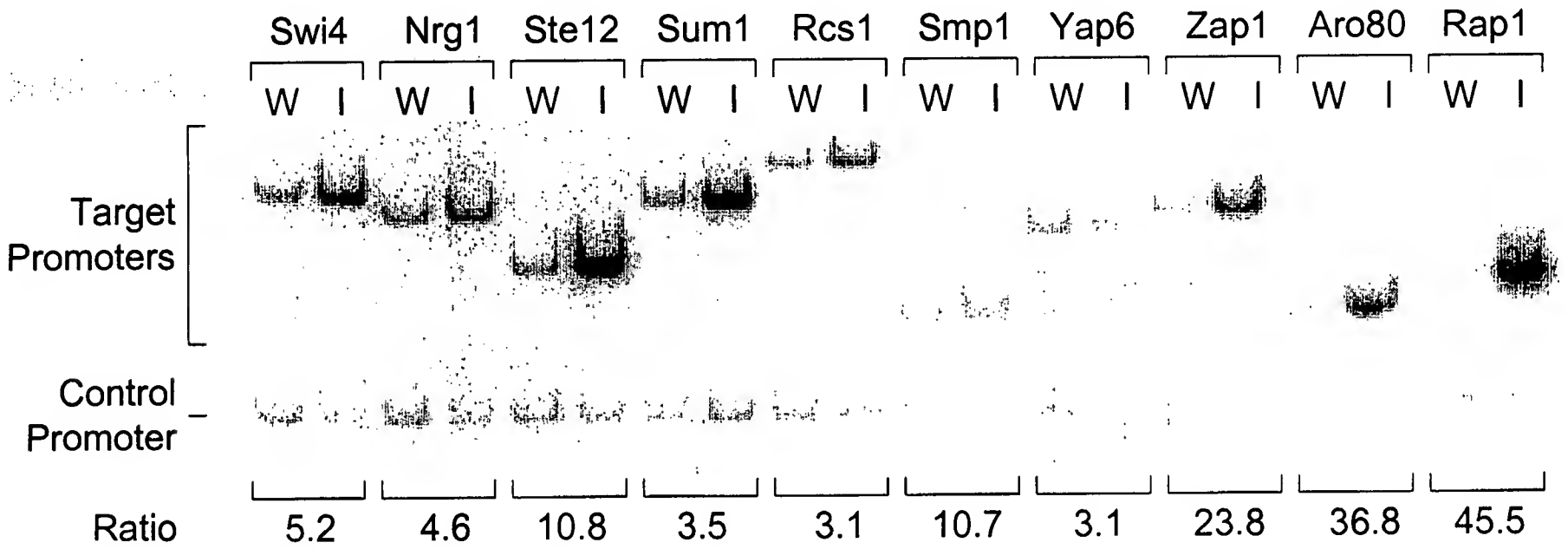
## Data Generation




## Error Model



# False Positive and False Negative Rates



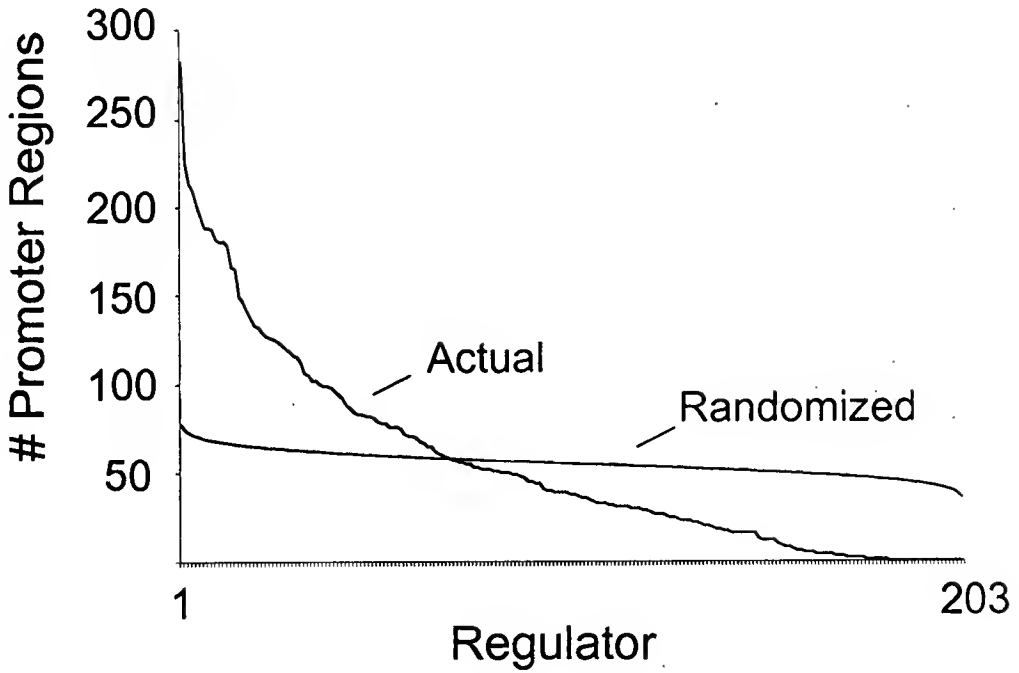

Using a p-value threshold of 0.001:

False positive rate ~6%
False negative rate ~30%

# Summary of Genome-wide Binding Data

## Promoter Regions Bound per Regulator

(High confidence: P < 0.001)




- Over 11,000 protein-DNA interactions
- Avg # promoter regions bound per regulator: 55
- Most abundant regulators bind to largest # promoters
- No promoter occupancy observed: 20 regulators

# Regulator Binding Site Sequence Discovery

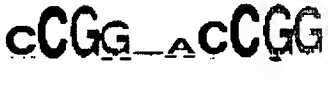
Re-discovered sequences:

Abf1 

Cbf1 

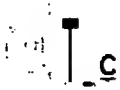
Gcn4 

Hsf1 

Leu3 

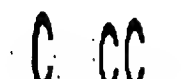
Ste12 

Newly discovered sequences:

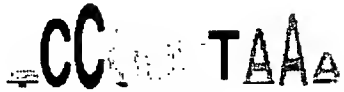
Aft2 

Cin5 

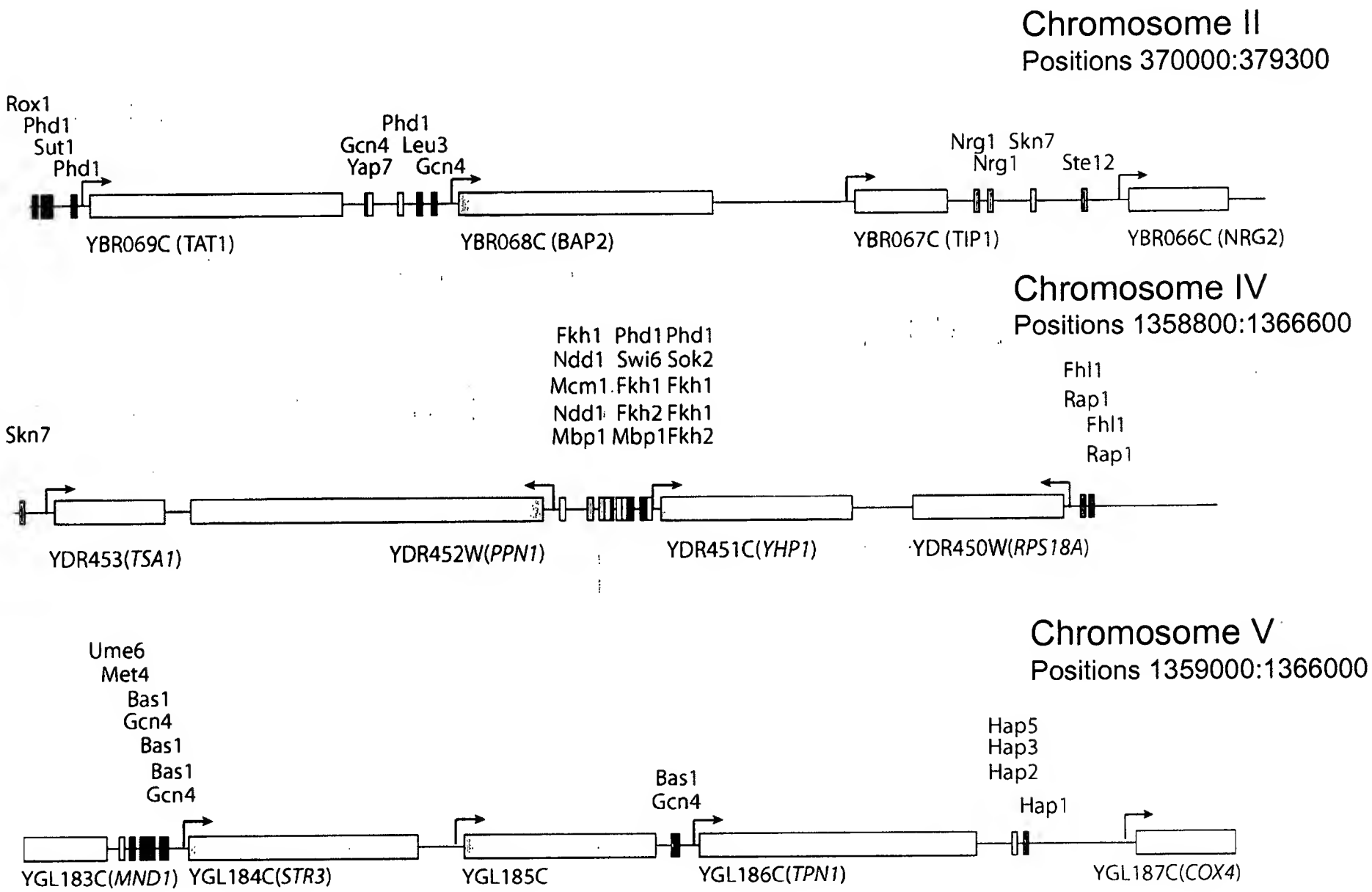
Phd1 

Rds1 

Stb5 

YDR026C 

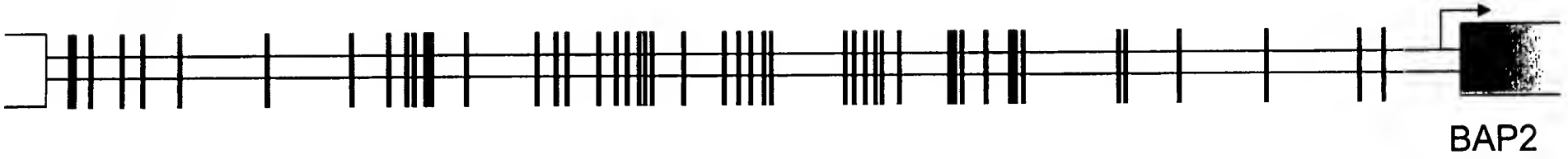
# Samples of the Draft Transcriptional Regulatory Code



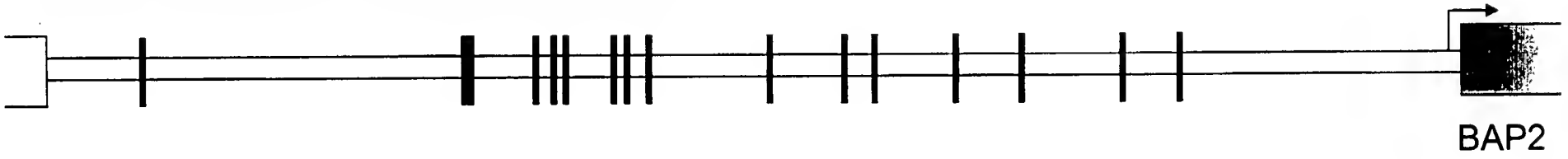
# Influence of Data Types on Transcriptional Regulatory Code Discovery

## Intergenic Promoter Region for BAP2

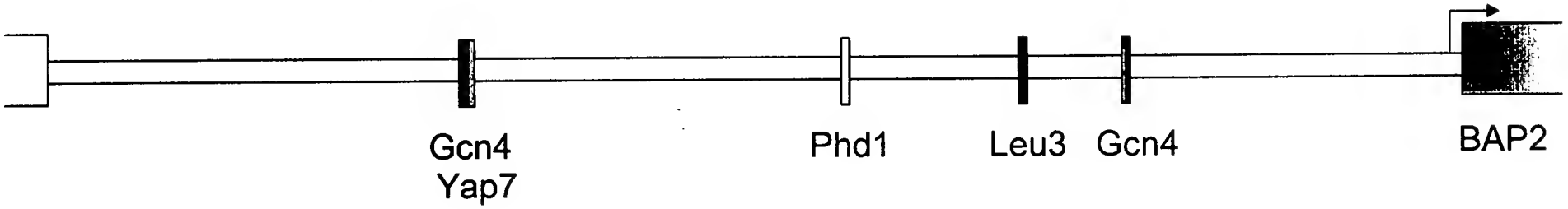
All Motifs



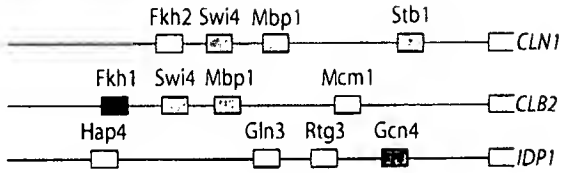

Motifs Conserved Across Multiple Species




Conserved Motifs AND Binding Information from Location Analysis



# A Draft Transcriptional Regulatory Code for Yeast

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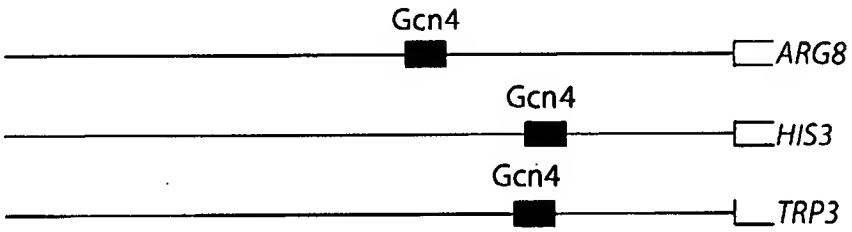
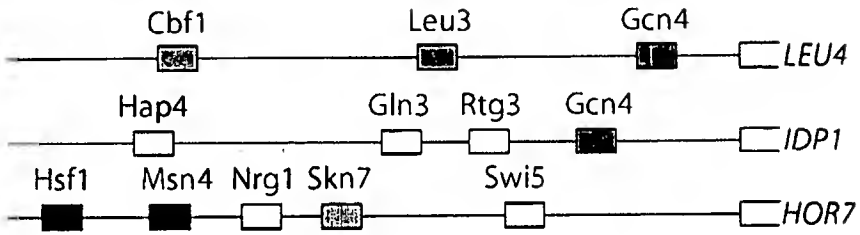
# Promoter Architectures

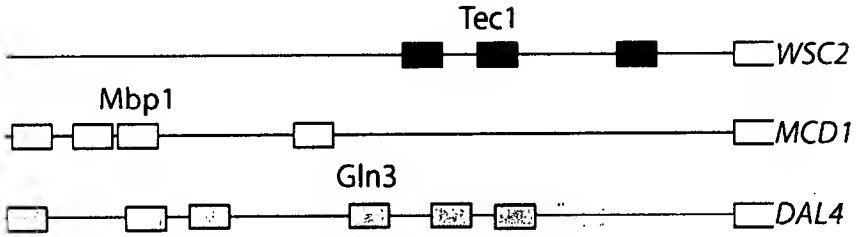
## Single regulator



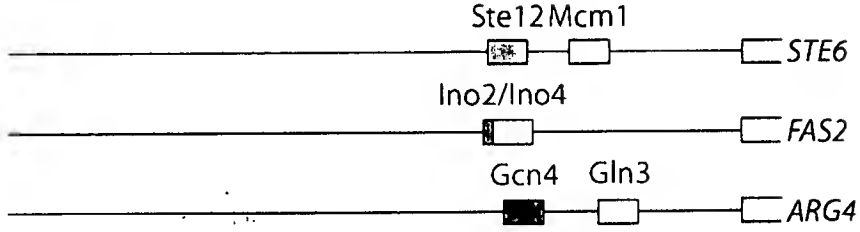

## Multiple regulators




## Repetitive motifs




## Co-occurring regulators



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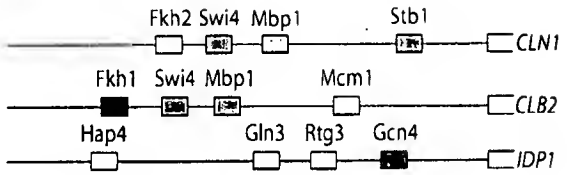


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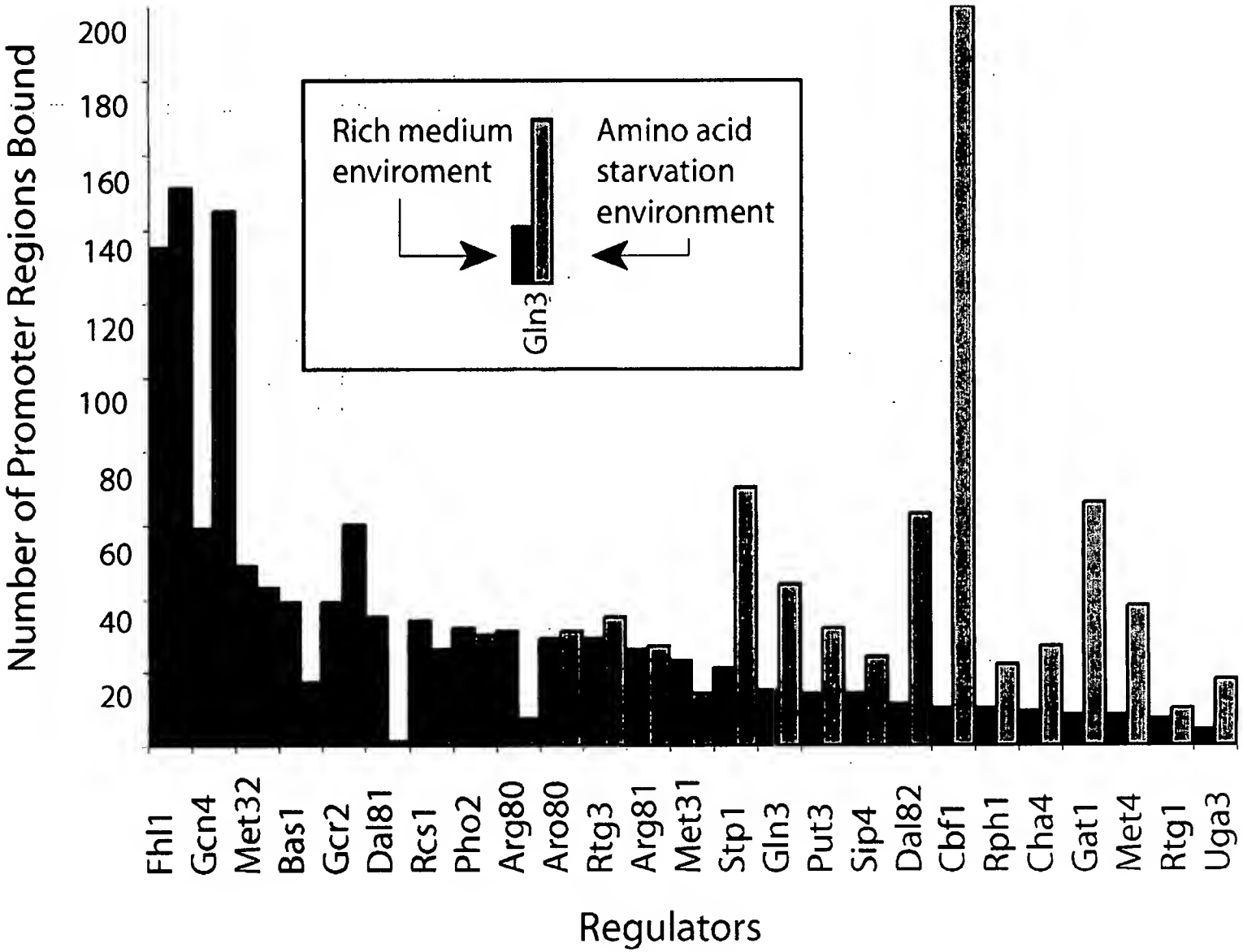
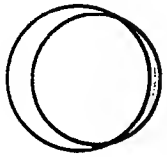
# Environmental Effects on Genomic Binding



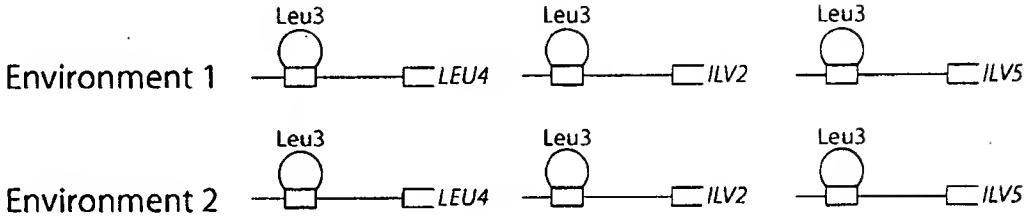
# Environment-Specific Regulator Behaviors

## Global Behavior

Condition Invariant (e.g. Leu3)



## Selected Regulator-Gene Interactions



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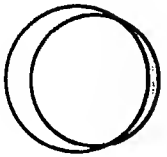
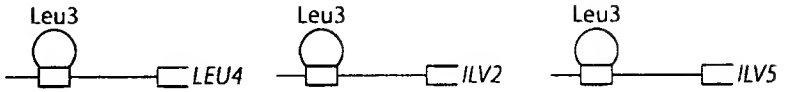
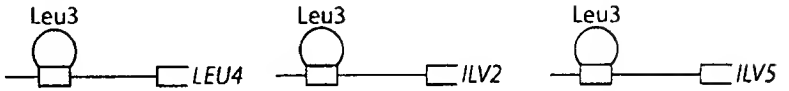


Environment 1

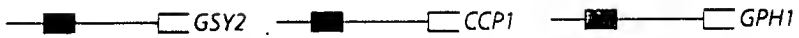



Environment 2

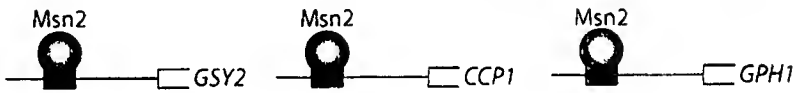



Condition Enabled (e.g. Msn2)

Environment 1




Environment 2

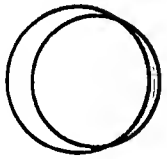


# Environment-Specific Regulator Behaviors

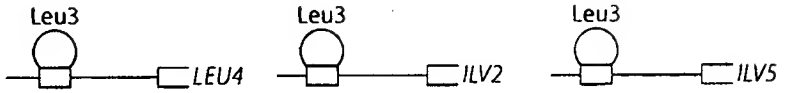
Global Behavior

Selected Regulator-Gene Interactions

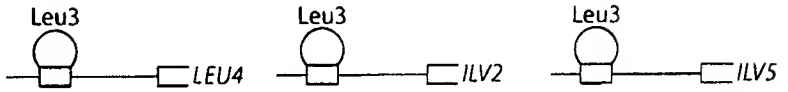
Condition Invariant (e.g. Leu3)



Environment 1



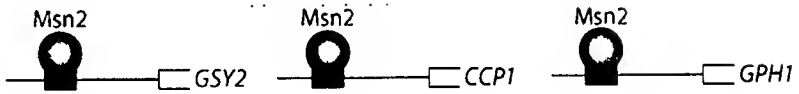

Environment 2




Condition Enabled (e.g. Msn2)

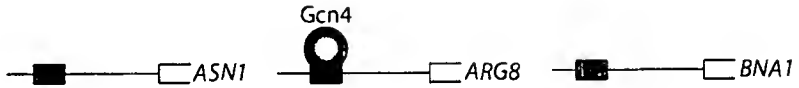
Environment 1


Environment 2

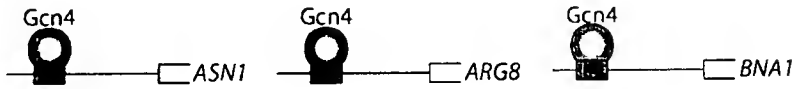



Condition Expanded (e.g. Gcn4)

Environment 1




Environment 2

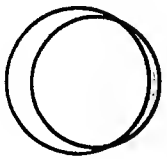


# Environment-Specific Regulator Behaviors

Global Behavior

Selected Regulator-Gene Interactions

Condition Invariant (e.g. Leu3)



Environment 1: Leu3 → *LEU4*; Leu3 → *ILV2*; Leu3 → *ILV5*

Environment 2: Leu3 → *LEU4*; Leu3 → *ILV2*; Leu3 → *ILV5*

Condition Enabled (e.g. Msn2)

Environment 1: *GSY2*; *CCP1*; *GPH1*

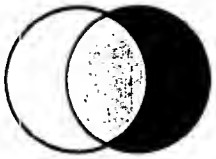
Environment 2: Msn2 → *GSY2*; Msn2 → *CCP1*; Msn2 → *GPH1*

Condition Expanded (e.g. Gcn4)

Environment 1: *ASN1*; Gcn4 → *ARG8*; *BNA1*

Environment 2: Gcn4 → *ASN1*; Gcn4 → *ARG8*; Gcn4 → *BNA1*

Condition Altered (e.g. Ste12)



Environment 1: Ste12 → *AGA1*; Ste12 → *STE12*; *MSB2*

Environment 2: *AGA1*; Ste12 → *STE12*; Ste12 → *MSB2*

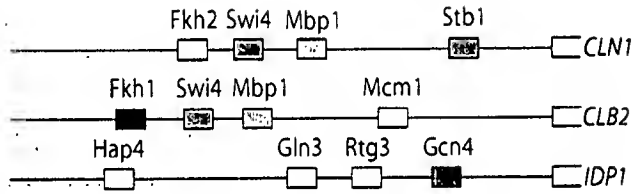
# Challenges for Future Drafts

- Additional data
  - More DNA-binding regulators
  - Chromatin regulators
  - More environmental conditions
- Tests for regulatory mechanisms
  - Regulator abundance, modifications and translocation
  - Integration with expression information
- Refined computational tools

# Regulation of Genome Expression in Health and Disease

## Yeast Transcriptional Regulatory Code

First draft developed using combination of
- Genome-wide regulator binding data
- Sequence conservation data



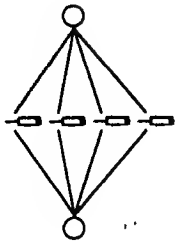

Ren et al. *Science* (2000)
Simon et al., *Cell* (2001)
Wyrick et al., *Science* (2001)
Lee et al., *Science* (2002)
Zeitlinger et al., *Cell* (2003)
Bar-Joseph et al., *Nature Biotech*nology (2003)

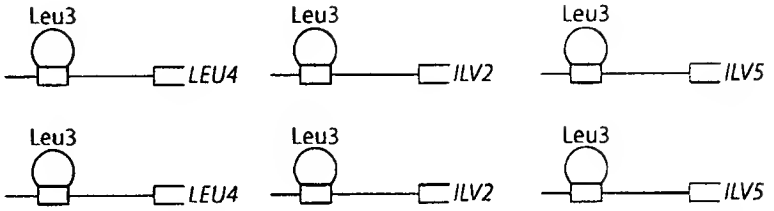
# Environment-Specific Regulator Behaviors

Global Behavior

Selected Regulator-Gene Interactions

Condition Invariant (e.g. Leu3)

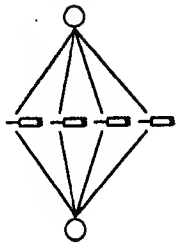




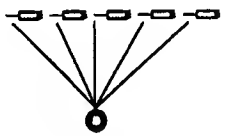
# Environment-Specific Regulator Behaviors

## Global Behavior

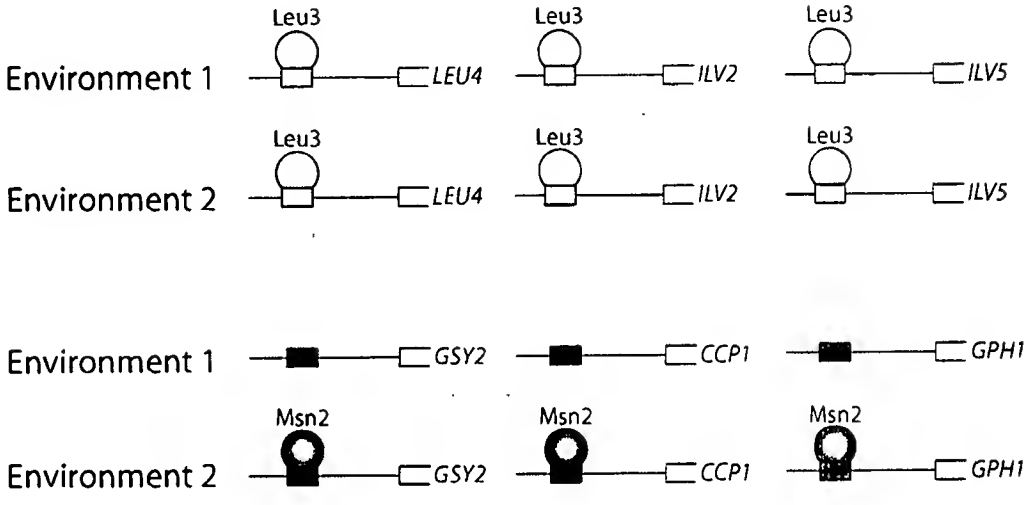
Condition Invariant (e.g. Leu3)



Condition Enabled (e.g. Msn2)



## Selected Regulator-Gene Interactions

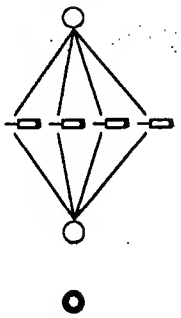


# Environment-Specific Regulator Behaviors

Global Behavior

Selected Regulator-Gene Interactions

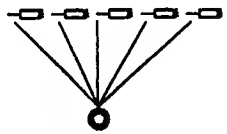
Condition Invariant (e.g. Leu3)



Environment 1: Leu3 → *LEU4*; Leu3 → *ILV2*; Leu3 → *ILV5*

Environment 2: Leu3 → *LEU4*; Leu3 → *ILV2*; Leu3 → *ILV5*

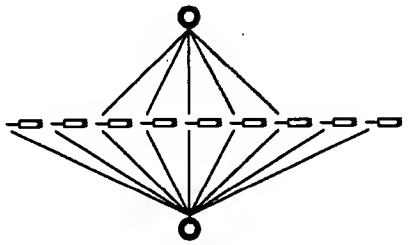
Condition Enabled (e.g. Msn2)



Environment 1: *GSY2*; *CCP1*; *GPH1*

Environment 2: Msn2 → *GSY2*; Msn2 → *CCP1*; Msn2 → *GPH1*

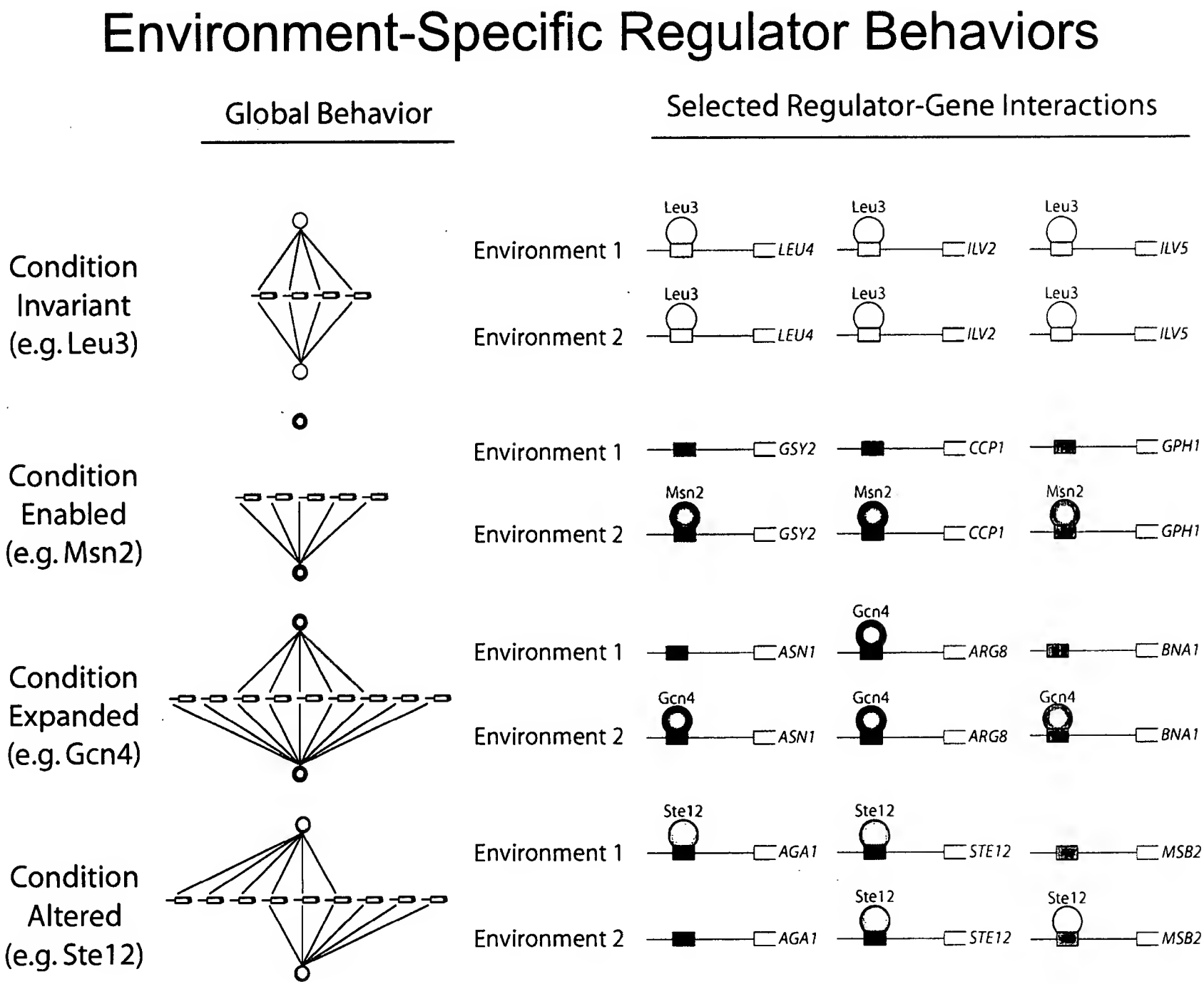
Condition Expanded (e.g. Gcn4)



Environment 1: *ASN1*; Gcn4 → *ARG8*; *BNA1*

Environment 2: Gcn4 → *ASN1*; Gcn4 → *ARG8*; Gcn4 → *BNA1*

# Environment-Specific Regulator Behaviors



## Supplemental Application Data Sheet

### Application Information

| | |
|---|---|
| Application number:: | Not Yet Assigned |
| Filing Date:: | 03/04/04 |
| Application Type:: | Provisional |
| Subject Matter:: | Utility |
| Suggested Group Art Unit:: | N/A |
| CD-ROM or CD-R?:: | None |
| Sequence submission?:: | None |
| Computer Readable Form (CRF)?:: | No |
| Title:: | TRANSCRIPTIONAL REGULATORY CODES OF EUKARYOTIC GENOMES AND METHODS THEREOF |
| Attorney Docket Number:: | WIBL-P60-035 |
| Request for Early Publication?:: | No |
| Request for Non-Publication?:: | No |
| Small Entity?:: | No |
| Petition included?:: | No |
| Secrecy Order in Parent Appl.?:: | No |

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| | |
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| Middle Name:: | T. |
| Family Name:: | Harbison |
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| State or Province of Residence:: | MA |
| Country of Residence:: | US |
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| | |
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| State or Province of mailing address:: | MA |
| Postal or Zip Code of mailing address:: | 02169 |

| | |
|---|---|
| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | US |
| Status:: | Full Capacity |
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| Middle Name:: | B. |
| Family Name:: | Gordon |
| City of Residence:: | Somerville |
| State or Province of Residence:: | MA |
| Country of Residence:: | US |
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| City of mailing address:: | Somerville |
| State or Province of mailing address:: | MA |
| Postal or Zip Code of mailing address:: | 02143 |

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| Applicant Authority Type:: | Inventor |
| Primary Citizenship Country:: | US |
| Status:: | Full Capacity |
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| Middle Name:: | A. |
| Family Name:: | Young |
| City of Residence:: | Weston |
| State or Province of Residence:: | MA |
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| Street of mailing address:: | 216 Highland Street |
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**Correspondence Information**

Correspondence Customer Number:: 28120

**Representative Information**

Representative Customer Number:: 28120

**Assignee Information**

| | |
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| Assignee name:: | Whitehead Institute for Biomedical Research |
| Street of mailing address:: | Nine Cambridge Center |
| City of mailing address:: | Cambridge |
| State or Province of mailing address:: | MA |
| Postal or Zip Code of mailing address:: | 02142-1479 |